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Transmammary Passage of *Strongyloides papillosus* in the Goat and Sheep[1]

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ABSTRACT: Filariform larvae of *Strongyloides papillosus* were found in milk of naturally or experimentally infected sheep or goats at irregular intervals up to 70 days postpartum. Although the filariform larvae recovered from milk grossly resembled filariform larvae cultured from feces, the former were longer, had longer and wider esophagi and genital primordia, and the distance from the genital primordium to the tip of the tail was shorter.

This observation helps to explain why *Strongyloides papillosus* is frequently the only nematode found in parasite-free lambs. Even though the level of passage may be irregular or even very low, given the right circumstances of poor husbandry and through repeated exposure, an acute parasitism may develop in the very young lamb or kid.

Olson and Lyons (1962) showed that larvae of *Uncinaria lucasi* were transmitted in the colostrum of the northern fur seal. Soon thereafter larvae of other nematodes were found in colostrum of their respective hosts: *Strongyloides ransomi* in the pig (Moncol and Batte, 1966), *Ancylostoma caninum* in the dog (Stone and Girardeau, 1966), *Toxocara canis* in the dog (Stone and Girardeau, 1967), *Strongyloides westeri* in the horse (Lyons et al., 1969), *Strongyloides papillosus* in the sheep and cow (Lyons et al., 1970), *Toxocara cati* in cats (Swerczek et al., 1971), and *Neoascaris vitulorum* in cattle (Warren, 1969).

The purpose of this study was to determine (a) if *Strongyloides papillosus* larvae occur in the milk of the goat, (b) for what duration are larvae passed; in what numbers and frequency both for the sheep and goat, and (c) if morphologic differences exist with the filariform larvae.

Materials and Methods

S. papillosus infection was known to exist in the pasture grazed by sheep and goats. This was determined by positive fecal egg counts in lambs and kids during the current grazing season. The infection was of short duration and of low magnitude. Ewes and does selected had negative fecal egg counts for *S. papillosus*. Therefore, additional exposure was employed. Each of three ewes was infected with *S. papillosus* in nature. Each of a second group of three ewes was given 1,400,000 larvae percutaneously (Turner, 1959) in fortnightly doses of 700,000 larvae, and one ewe was given a single subcutaneous injection of 1,000,000 larvae. Each of three does was infected percutaneously (Turner, 1959) with 1,250,000, 200,000, or 700,000 larvae, respectively.

Dated pregnancies existed for ewes, but the does were pasture-bred.

Animals were infected at midgestation, with the exception of doe #166 which was infected during the last 4 weeks of gestation.

As soon as the young were born, the offspring and mother were placed in individual suspended wire-bottom cages. Each night the lambs or kids were removed from the mother. The following morning, all of the milk was collected from one-half of the udder before the offspring were returned to the mother to suckle. This procedure was followed until a minimum

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Table 1. Recovery of *S. papillosus* from milk of sheep or goats.

Animal No.	Method of exposure	Total larvae given	No. days milked	Total larvae in milk	Day 1st seen	Day Last seen
Ewe 2	Natural		21	Neg.		
Ewe 97	Natural		21	Neg.		
Ewe 29	Natural		29	2	18th	19th
Ewe 78	Percutaneous	1,400,000	21	Neg.		
Ewe 8	Percutaneous	1,400,000	91	199	3rd	70th
Ewe 111	Percutaneous	1,400,000	18	Neg.		
Ewe 34	S/Q injection	1,000,000	61	7	8th	40th
Goat 183	Percutaneous	1,250,000	22	Neg.		
Goat 160	Percutaneous	200,000	19	2	5th	5th
Goat 166	Percutaneous	700,000	15	Neg.		

of 14 consecutive negative samples were obtained from an individual animal. Milk volume was recorded and the entire sample was placed in a Baermann funnel for 6 to 8 hr. The contents of the collecting tube were further filtered through a Millipore prefilter No. AP-200 2500 (Millipore Filter Corp., Bedford, Mass.). The larvae were identified, counted, and a portion measured for morphological characteristics. Cultured filariform larvae were obtained by baermannization of a fecal–peat moss mixture incubated at room temperature (23 C) for at least 10 days.

Measurements of milk-passed and cultured filariform larvae were analyzed statistically.

Results

Only one of the three does passed larvae in the milk. Doe #160, given 200,000 larvae 40 days prepartum, passed two larvae the 5th day postpartum. No larvae were seen during the next 14 days.

One larva was recovered the 18th and one the 19th day postpartum from a control ewe (29). Ewe #34, given 1,000,000 S. *papillosus* larvae subcutaneously 94 days prepartum, passed a total of seven larvae between the 8th and the 40th days postpartum. Ewe #8, given two doses each of 700,000 larvae percutaneously, sporadically passed a total of 199 larvae between the 3rd and 70th days postpartum. The first 18 days, the number of larvae recovered varied from day to day with passage occurring on 4 separate days. Larvae were then recovered daily for the next 23 days after which the original pattern of irregular appearance of larvae resumed. The number of larvae recovered ranged from 1 to 31 per day. The greatest number of larvae, 31, were recovered the 31st day. The great majority of larvae were recovered 19 to 41 days postpartum.

Morphologically, larvae recovered from milk had a trfiurcated tail, but differed significantly from the filariform larvae recovered from cultured feces (Table 2). The genital primordium of filariform larvae from feces was quite indistinct, giving an appearance of being in the one- to two-cell stage, whereas that of larvae from milk appeared to be in a four- to eight-cell stage.

The filariform larvae recovered from milk were longer, had longer and wider esophagi, genital primordium, and the distance from the genital primordium to the tip of the tail was shorter.

Discussion

Lyons et al. (1970) reported recovery of one to four larvae of S. *papillosus* in milk of ewes between 8 and 19 days postpartum.

Table 2. Average measurements (μ) of milk-derived and coprologically cultured larvae of *Strongyloides papillosus*.

	Larvae from Milk	Culture 18th day	Coeff. variance (%)
Total length	589.1*	569.4	6.2
Length of esophagus	268.8*	231.9	6.4
Width of esophagus	18.9*	15.1	7.8
Length of tail	8.6*	8.0	8.6
Length of genital primordium	18.8*	10.6	19.4
Width of genital primordium	7.8*	3.2	20.9
Distance of genital primordium to tip of tail	221.6*	233.4	8.3
Number of larvae measured	50	50	

* $P < .01$.

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In the present study, larvae appeared earlier (day 3) and persisted longer (70 days) than in Lyons et al. (1970). The appearance of 31 larvae in milk from one-half of the udder also surpasses previous reports. The time of first appearance, frequency of appearance, and duration of appearance was extremely variable among individuals.

The phenomenon of milk transmission does not appear to be of major importance in the perpetuation of the species *S. papillosus* in sheep and goats. However, compared to *S. ransomi* (Moncol, unpublished observation), in which case larvae are far more abundant in sow colostrum (first 48 hr), transmammary passage of *S. papillosus* could be considered a far less important mode of transmission.

As for the sheep and goat, the location of larvae prior to parturition remains undetermined. *S. ransomi* has been observed in and isolated from subcutaneous adipose tissue of the pig (Moncol, unpublished observation). The pig has abundant subcutaneous adipose stores and *S. ransomi* has adapted to and remains in this tissue to await parturition. Sheep and goats do not generally have abundant adipose tissue along the ventral abdomen. Rather large amounts may be present dorsally and internally about the omentum and mesentery. Turner et al. (1960) observed significant numbers of *S. papillosus* in venous circulation following percutaneous infection. Likewise, large numbers of larvae were demonstrated in the gracilis muscle up to 136 hr after percutaneous exposure. It would appear that *S. papillosus* possess great ability to migrate within tissue at rather distant sites from the point of entry. On the other hand, *S. ransomi* seems to have a greater predilection for adipose tissue and is seldom found in muscular tissue.

The sheep and pig differ somewhat in their husbandry. Whereas the pig is confined to smaller quarters and frequently becomes coated with its own excreta, the sheep and goat are pastured animals and will only contaminate their exteriors accidentally. The possibility does exist with sheep in that occasionally the wool about the perineum becomes soiled with excreta, especially if prolonged diarrhea exists. This accumulated excrement continues to build up and remains moistened. Ample heat is radiated from the skin to provide a suitable culturing mechanism. Thus, a constant source of infective *Strongyloides* larvae could be present for percutaneous infection. It is common to find an intestinal infection existing in the sheep or goat at parturition, but this is rarely observed in the sow. The sow does not begin storing larvae in adipose tissue until a degree of sensitization has occurred following initial exposure, which may be a period of time as short as 3 weeks. The sheep and goat may not be exposed to large numbers of infective *Strongyloides* larvae as is the case with the sow. Consequently, sensitization may be slower in developing, if at all. Round (1963) observed that under constant pasture exposure, fecal egg counts of *S. papillosus* persisted at a low level for several months. Under a constant source of infection, the pig will lose its intestinal infection of *S. ransomi* in 12 to 15 weeks (Moncol, unpublished observation).

From gross observation, the milk-passed larvae resembled filariform larvae with two notable exceptions. Milk-passed larvae did not possess the degree of activity, were much more sluggish, and the genital primordium was easily recognized. The results of the present study confirm those of Basir (1950) as to total length, esophageal length, and width of infective larvae. This author's measurement of the genital primordium (25 μ) is difficult to compare with those obtained in the present study.

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Hydroxyl Ion, an Attractant to the Male of *Pelodera strongyloides*

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ABSTRACT: Both male and female sexes of *Pelodera strongyloides* produce OH^- as pulsed phenomena which may attract the male but probably not the female sex. Both sexes may each produce other as yet unidentified factor(s) which may be involved in the behavioral physiology of this worm. These data indicate that therapeutic agents specifically designed to block nematode attractant(s) could significantly decrease the reproductive potential of the parasite.

Pheromones—substances produced by an organism which influence the behavior of other members of the same species—have been identified in many organisms. Because nematodes are economically and medically important parasites of a variety of hosts, it is valuable to determine if attractant(s) are important to their behavioral physiology. The present work adds knowledge to that acquired by some other investigators (Beaver, 1964; Greet, 1964; Roche, 1966; Bonner and Etges, 1967; Marchant, 1970; Salm and Fried, 1973; Ward, 1973) and uses *Pelodera strongyloides*, the dioecious, nonparthenogenetic, facultative parasite that has been implicated by numerous authors as a secondary invader in dermatitis infections (Dikmans, 1948; Levine et al., 1950).

Materials and Methods

The following general methods were used to study attraction with *P. strongyloides*. Males and virgin females were identified according to descriptions of Chitwood and Chitwood (1950), Osche (1952, 1954), and Goodey (1951). Worms from eggs from a single nonparthenogenetic female obtained from cultures maintained at the Animal Parasitology Institute were cultured on standard Difco nutrient agar at room temperature (25 to 27 C). Females, chosen at a size and age before they would have normally mated, were considered virgin by the absence of a copulatory plug or cement-sperm remnant. The bacterial associates which accompanied every phase of each experiment were not identified.

An isolation apparatus was constructed by placing a piece of clear, polyethylene tubing (inside diameter: 3 mm) 2 mm high on a circular 6-mm paper cutout previously soaked in melted nutrient agar (Fig. 1A). An isolation apparatus was placed at each of four posts on nutrient agar 1 cm deep in a petri plate, each post being 20 mm from the center of the plate. Twenty-four hours before testing, male and female worms were separated from each other by placing them on separate nutrient agar petri

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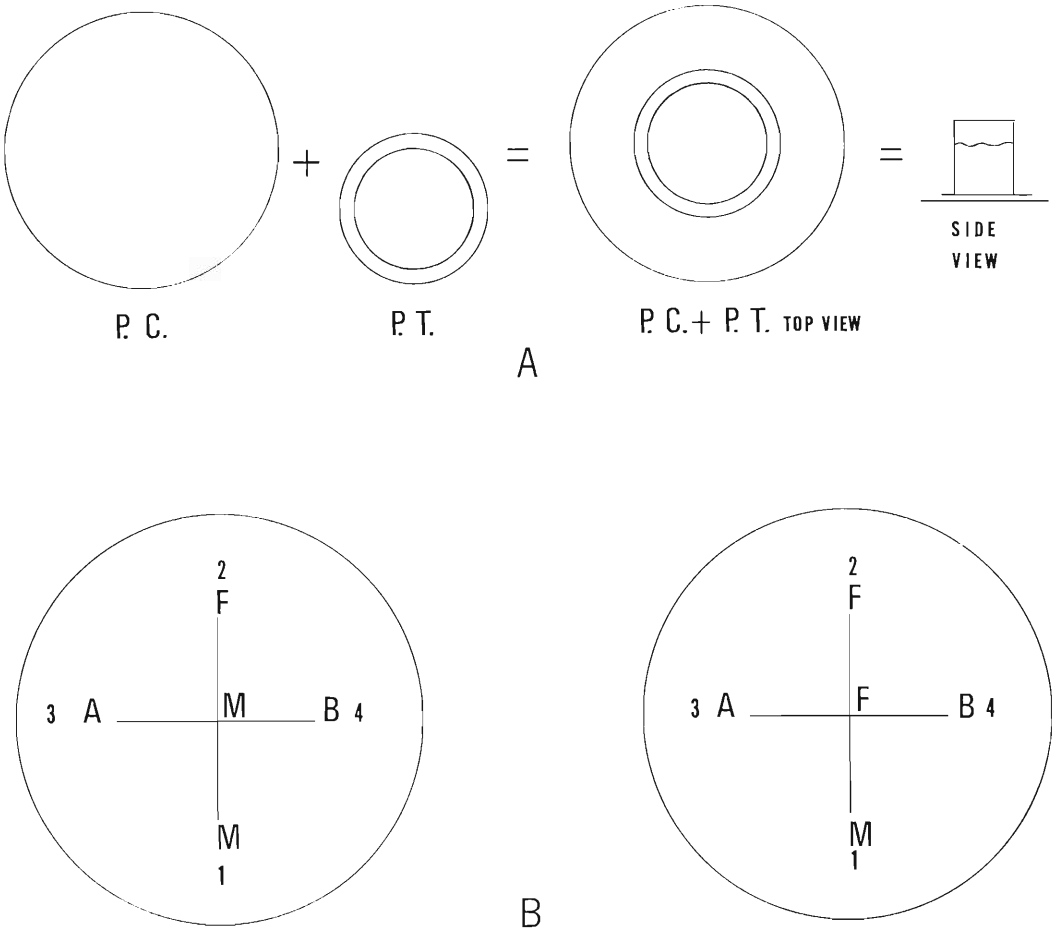


Figure 1, A, B. A, Isolation apparatus. P.C., paper cutout; P.T., polyethylene tubing. B, Experimental design.

plates. One viable male and a virgin female were placed separately in isolation at posts 1 and 2, whereas posts 3 and 4 contained no nematodes but were inoculated with their associated bacteria (Fig. 1B). Bacterial associates were also grown separately from the worms by culturing nutrient agar stabs of the bacteria at room temperature. A single worm of a selected sex was placed at the center of the agar plate and allowed to migrate freely. It usually took an hour to set up each experiment and 5 min to place the worms which were free to migrate in place. A plus was scored if the worm tagged any one of the four posts. Adjacent plates were reversed and observed continuously for 6 hr. Fifty worms of each sex were tested (five groups consisting of 10 plates each; total: 100 samples) (Figs. 2A, B).

A companion experiment was run to determine the migration pattern of each sex when the attractant(s) were mechanically blocked with paraffin and the results were compared with those obtained from the first section of this experiment. Forty-seven of each sex were tested (five groups consisting of 10 plates each; total: 100: three sets had to be discarded). The paper cutouts were soaked in melted paraffin and the clear polyethylene tubing

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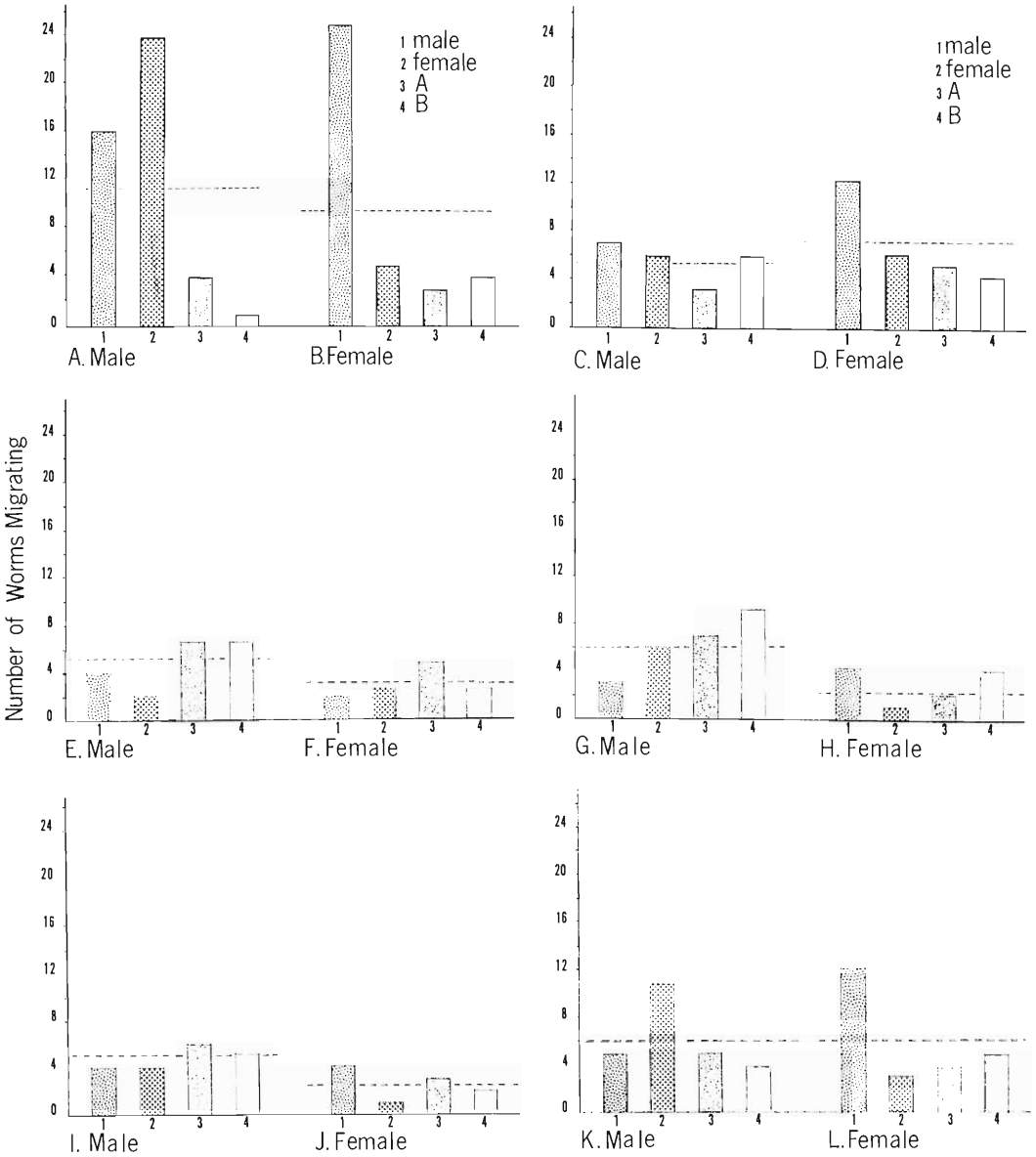


Figure 2, A–L. Histograms: A, The male attracted to the male and female as opposed to bacterial controls. B, The female attracted to the male rather than the female and bacterial controls. C, Insignificant attraction of male to isolation apparatus sealed with paraffin with male, female, and bacterial controls in place. D, Significant attraction of female to male with design as in C. E, F, Male and female worms equally attracted to all uninoculated isolation apparatus. G, H, Male and female worms equally attracted to all isolation apparatus inoculated with bacteria only. I, J, Male and female worms equally attracted to all posts inoculated with bacteria only with phosphate buffer incorporated into nutrient agar. K, L, Effect of phosphate buffer on decreasing the ability of the male to find both male and female and the female to find the male.

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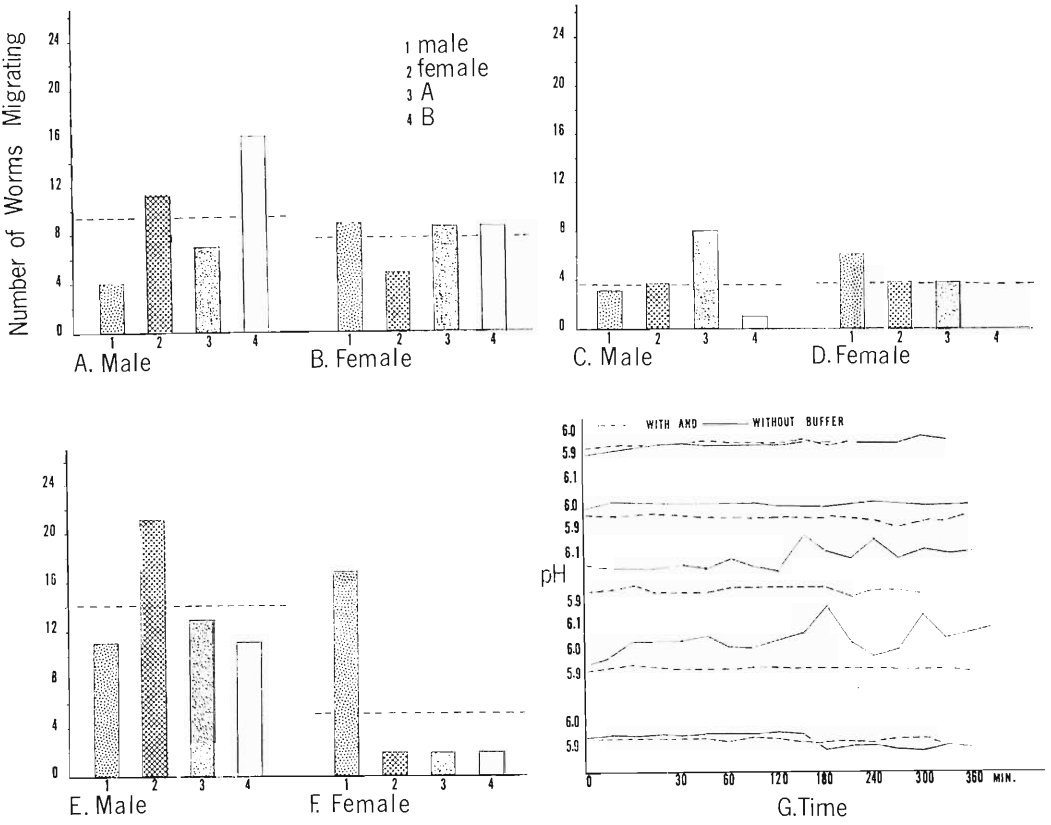


Figure 3, A–G. A, Male attracted to an alkaline pH source rather than male, female, or bacterial controls. B, Female not attracted to the above alkaline pH source. C, D, Inability of the male or female to find alkaline pH source when phosphate buffer is incorporated into the agar. E, F, "Masking effect" on males and not females when pH of nutrient agar is 9.0 (compare with 2A, B). G, pH measurements in microenvironment of worms. Top to bottom: with and without phosphate buffer incorporated into agar: nutrient agar blank, bacterial control, 200 males, 200 females, 100 males: 100 females.

applied to them thereby creating a water-insoluble mechanical barrier between the worm and nutrient agar (Figs. 2C, D). Other control experiments with the same basic design included: (1) no bacteria were placed at any of the above posts (Figs. 2E, F), and (2) bacteria were placed at all of the above posts without (Figs. 2G, H) and with (2I, J) 0.1 M phosphate buffer incorporated into the agar (pH 6.8 to 7.0).

The literature on chemical attraction in nematodes, well summarized by Croll (1970), as well as my own observations, indicated that pH could be an important factor in this nematode's behavioral physiology. With the same basic design, the previous experiment was repeated but with 0.1 M phosphate buffer incorporated into the nutrient agar (2K, L). Further experiments were run with 0.1 M $NaHCO_3$ in nutrient agar placed at post B with A remaining blank (Figs. 3A, B). This experiment was repeated with 0.1 M phosphate buffer incorporated into the nutrient agar (Figs. 3C, D). The basic design was tested for a "masking effect" by adjusting the nutrient agar to pH 9.0 with 0.1 M NaOH (Figs. 3E, F). The alkaline pH source (0.1 M $NaHCO_3$, pH 8.4) and phosphate buffer were selected according to the following criteria: (1) $NaHCO_3$ did not diffuse rapidly from the agar block as did

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NaOH and KOH providing a continuous alkaline pH over the period of observation; (2) maximum buffering capacity was achieved with phosphate buffer at pH of 6.8; and (3) the concentration of the Na^+ from both the $NaHCO_3$ and phosphate buffer system gave no gradients of Na^+.

The above experiments were tested with chi-square at the 0.05 level of significance at 3 degrees of freedom. Expected values were calculated from the data of each figure by dividing the total number of worms that tagged a post by 4. These values for each set of data are represented by the horizontal dotted lines in Figures 2 and 3.

The following experiments were run to determine if *P. strongyloides* influenced the pH of its microenvironment. Plain nutrient agar petri plates (pH 5.9 to 6.0) were each incubated with *P. strongyloides* in the isolation apparatus. Electrometric determinations for pH were made with the Fisher Model 520 meter using precision buffers. Measurements were recorded at 5-min intervals during the first hour, 15-min intervals for the second hour, and every half hour thereafter for 6 hr. The first group of nutrient agar plates contained 0.1 M phosphate buffer incorporated into them at pH 5.9 to 6.0 and the second group contained no buffer at the same pH (Fig. 3G). The following plates were run: nutrient agar blank, bacterial control, male (200) plus bacteria, female (200) plus bacteria, male (100) and female (100) plus bacteria. Large numbers of worms were used because even the Model 520 meter could not measure pH fluctuations in the microenvironment of a single worm.

Results

Forty-five (90%) of 50 males migrated to all posts (Fig. 2A). The males migrated more times to the male and female than to posts 3 or 4. Thirty-seven (74%) of the females migrated to all posts (Fig. 2B). The females migrated more times to the male than to any of the other posts. When the attractant(s) were mechanically blocked with paraffin, 22 (47%) males migrated about equally to all posts (Fig. 2C), and 27 (57%) of 47 females migrated to all posts (Fig. 2D). The females showed some preference for the male as compared with the other three posts. When an isolation apparatus only was at the posts, 19 (38%) of 50 males (Fig. 2E) and 13 (26%) of 50 females migrated to all posts (Fig. 2F). Neither the male nor the female showed a preference for any one post. When bacteria only without buffer were at each of the four posts, 25 (50%) of 50 males (Fig. 2G) and 11 (22%) (Fig. 2H) of 50 females migrated to all posts. When bacteria only with buffer were at each of the four posts, 19 (38%) of 50 males (Fig. 2I) and 10 (20%) of 50 females (Fig. 2J) migrated to all posts. Neither male nor female showed a preference for any one post (Figs. 2E–J). When 0.1 M phosphate buffer was incorporated into nutrient agar using the original design, 25 (50%) of 50 males and 24 (48%) of 50 females migrated to all posts (Figs. 2K, L). The male showed a preference for the female (Fig. 2K) which showed a preference for the male (Fig. 2L). Thirty-eight (76%) of 50 males and 31 (62%) of 50 females migrated to all posts (Figs. 3A, B) when 0.1 M $NaHCO_3$ was used as an alkaline pH source. When the experiment was repeated with phosphate buffer incorporated into the agar, 16 (32%) of 50 males and 14 (28%) of 50 females migrated to all posts. Neither males nor females were attracted to the alkaline pH source (Figs. 3C, D). When the pH of the nutrient agar was elevated to 9.0, 48 (74%) of 65 males and 21 (32%) of 65 females migrated to all posts (Figs. 3E, F). The males did not migrate more to the females as compared with the other posts than what might be expected from random migration. The females showed a significant preference for the males.

No obvious differences were detected between the controls and experimentals when phosphate buffer was and was not incorporated into the nutrient agar (Fig. 3G). The bacterial controls were similar to the nutrient agar blank with little alteration of the pH of the microenvironment over the period of time that measurements were made. The males and females definitely altered the pH of their microenvironments; and combinations of both male and female gave variable results. The standard error of the mean for these measurements was $\pm$ 0.004 units.

Discussion

Figs. 2A–3G clearly show the following relationships: (1) the male is attracted to both

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sexes of *P. strongyloides* (Fig. 2A), whereas the female is attracted only to the male (Fig. 2B); (2) the attractant produced by the male may be blocked either mechanically (Fig. 2C) or with phosphate buffer (Fig. 2K); (3) the presence of the unidentified bacteria did not noticeably influence the migration of the worms (Figs. 2E–H); (4) other than interfering with the worm's ability to find a post, the phosphate buffer had no detectable influence on worm behavior (Figs. 2G–J); (5) the male but not the female was attracted to the alkaline pH source at post B (Figs. 3A, B); (6) the alkaline pH source was successfully blocked by the phosphate buffer indicating that OH^-, rather than Na^+, is the attractant (Figs. 3C, D); (7) a high pH (9.0) causes a "masking effect" with regard to the male but not to the female (Figs. 3E, F); (8) the males and females produce OH^- as pulsed phenomena which are effectively blocked with phosphate buffer (Fig. 3G). That the worms showed the usual behavior and morphology when cultured with phosphate buffer indicated that it affected only the worm's ability to find another male or female. The phosphate buffer block shows that if the attractant (OH^-) is selectively bound then the reproductive potential of the parasite may be reduced; e.g., parasites that cannot find one another cannot mate. Hopefully this will lead to isolation or synthesis of inhibitors specifically designed to block specific pheromones produced by gastrointestinal nematode parasites.

The exteroreceptor(s), whatever and wherever they may be, are sensitive to specific changes in their environment. The OH^- created by the worms as pulsed phenomena are well suited as a stimulus and may serve simply as a general attractant. Because the data indicate the possibility of more than one type of attractant (Figs. 2K, L), it can be postulated that there may be more than one exteroreceptor. Samoiloff et al. (1973) believe that the receptors for sex attractant(s) are at the tip of the spicule. Ward (1973) believes that these receptors are at the anterior end.

Ward (1973) believes *C. elegans*, which feeds on bacteria, is attracted to them because they secrete cAMP. The results of this study do not indicate this, because *P. strongyloides* was no more attracted to isolation chambers in which bacteria were inoculated than to those which had not been so inoculated (Figs. 2E–H). These data also indicate that the presence of unidentified bacteria used in these experiments did not influence the results.

The female probably does not respond appreciably to OH^-. Figure 2L shows that the female significantly orients to the male, whereas the male, although it orients to the female, does not orient beyond what might be expected (Fig. 2K). The female does not significantly orient to an alkaline pH source, whereas the male does (Figs. 3A, B). The female is significantly oriented to the male in spite of attempts to "mask" the attractant, whereas the male does not significantly orient beyond what might be expected (Figs. 3E, F). The female, however, is blocked from the alkaline pH source by buffer but not significantly (Fig. 3D). These conclusions are based on calculated chi-square values which do (do not) significantly differ from those which might be expected from random migration. Furthermore, if a tray with a pH range of 5 to 9 is constructed and males and females are allowed to migrate freely from points pH 5, 7, and 9, the males aggregate to the alkaline side, whereas the females prefer a neutral environment (unpublished data).

The data reported herein partially explain aspects of the biology of *P. strongyloides*. This worm may be isolated in the field from fresh dung (cattle dung, pH 7.3 to 7.4). The males are probably attracted to the dung because of its alkalinity. Because OH^- may be a general attractant, they may function partially in aggregation. To my knowledge, OH^- is the simplest pheromone yet detected in either the animal or plant kingdom.

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Two Permanent Mounting Methods Compared After Six Years

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ABSTRACT: A comparison of morphometric and visual characteristics of *Xiphinema macrostylum* females is made 6 years after preservation in lactophenol or glycerine. Body length decreased in both preservatives. Esophagus length increased in lactophenol and decreased in glycerine. The guide ring appeared double in all glycerine-processed specimens and single in all lactophenol-processed forms.

A number of articles have been written concerning methods of killing and preserving nematodes, but none have been noted by the writer which compare the effects of fixation over long time periods. The primary objective in this study was to compare two methods of permanent fixation with each other and with the original data recorded 6 years earlier.

Methods

In February 1961 21 specimens of *Xiphinema macrostylum* Esser, 1966, were killed in 2% formalin (25 C) and mounted on 21 temporary slides. Specimens killed in 2% formalin die slowly and physical changes are not obvious until 20–30 min following cessation of movement. Distortion or shrinkage was not observed in these specimens. All measurements and camera lucida drawings essential to a species description were complete within 24 hr and permanent fixing of the specimens commenced.

Ten specimens (females and larvae) were fixed permanently 24 hr after killing, utilizing Baker's (1953) "Rapid Method for Mounting Nematodes in Glycerine." The remaining 11 specimens were placed in watch glasses in a solution of 3% formaldehyde, 4% lactophenol, and acid fuchsin. After most of this solution had evaporated fresh solution was added. Evaporation and addition of solution was repeated two more times. The specimens were then placed in a drop of undiluted lactophenol. Cover slip supports were placed in the mountant in both techniques and the cover slip was sealed with zut. Six years and 1 month later five females preserved by the lactophenol method and five by Baker's glycerine technique

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Table 1. Biometric comparison of two methods after 6 years of preservation.

	Lactophenol (3/67)		Glycerine (3/67)		Original description measurements (2/61) in 2% formalin	
	Range	Mean	Range	Mean	Range	Mean
Specimen length	2,100–2,430	2,282	2,010–2,250	2,180	2,150–2,480	2,340
"Alpha"	21.5–29.1	24.8	26.2–31	27	24–33	28
Width	79.8–101.8	93.4	72.2–89.2	80.8	70–94.5	84.8
Esophageal length	480–590	532	450–520	498	400–560	508
"Beta"	3.9–4.6	4.3	4.2–4.6	4.4	4–6	4.6
Vulva	950–1,010	980	880–960	930	890–1,220	996
Vulva per cent	40.7–45.2	42.6	43.7–44.8	42.6	39–51	43
Tail	31.2–42	34.3	31.5–37.8	34.4	35–55	44.2
"Gamma"	57.9–74.6	62.6	59.5–68.7	63.4	41–71	48

were remeasured and evaluated (all females examined lay in a lateral position).

LENGTH: Shrinkage in length occurred after 6 years using either method. Measurements of two lactophenol-processed specimens were compared with original specific measurements and a mean shrinkage of 10 μ in length was noted. Two glycerine-processed specimens by contrast had a mean shrinkage of 85 μ.

WIDTH: The original mean width (Table 1) was 84.8 μ: glycerine-processed specimens fell below this with a mean of 80.8 μ. Lactophenol-processed specimens were above the original mean with a mean of 93.4 μ. It was noted when transferring the specimens from the diluted lactophenol series to undiluted lactophenol that their bodies were flattened and had collapsed during the fixing schedule.

"ALPHA" (length/width): The flattened bodies of the lactophenol-processed specimens resulted in a smaller mean "alpha" of 24.8 (Table 1). The glycerine-processed specimens had a mean "alpha" of 27 which was very close to the original mean "alpha" of 28.

ESOPHAGUS: The length of the esophagus was measured from the oral aperture to the base (Table 1). Lactophenol-processed specimens exceeded the original mean length of 508 μ with a mean length of 532 μ. Glycerine-processed specimens had a mean of 498 μ indicating some esophageal shrinkage. A comparison with the original specimens showed one lactophenol-processed specimen with the same esophageal length, one with a 1-μ loss, and a third with a 44-μ loss. One glycerine-processed specimen showed a 12-μ loss while another showed a 6-μ gain in length.

"BETA" (length/esophagus length): Comparatively similar.

GUIDE RING: Guide ring position was comparatively similar. The guide ring appeared single in all lactophenol-processed specimens and double in all glycerine-processed specimens.

VULVA: Distance from the oral aperture to the vulva was least in the glycerine-processed specimens with a mean of 930 μ (Table 1). The lactophenol-processed specimens had a mean of 980 μ and the original specimens a mean of 996 μ. In five remeasured specimens (original specific specimens remeasured after 6 years), three lactophenol specimens had a mean shrinkage of 36 μ each, and two glycerine specimens had a mean shrinkage of 15 μ each. All five had shrunk with a maximum of 50 μ in one specimen.

VULVA PER CENT (length/vulva distance from head): Comparatively similar.

TAIL: Little difference in tail length was shown between lactophenol- and glycerine-processed specimens (Table 1). The original specimens, however, had a longer tail and greater mean.

"GAMMA" (body length/tail length): "Gamma" means were similar in the fixed specimens but quite different from the original "gamma" mean.

Specimens from both fixing methods were not too dissimilar on general appearances. Definition of the stylet parts and esophagus was slightly better defined in lactophenol-processed specimens. The nerve ring was obscure in one lactophenol-processed specimen. Intestinal cells were better defined in the

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glycerine-processed specimens. Gonads were not well defined in either mountant, but the glycerine-processed gonads were better defined than the lactophenol-processed gonads.

Discussion

Taxonomic descriptions are based on live specimens as well as those studied after a wide variety of killing, fixing, and preserving techniques. Unfortunately many workers describing new species fail to record the method which was used to kill, fix, or preserve the nematodes described. Sometimes when the methods are listed they fail to include the stage of fixation where the biometric or descriptive data originated. A random check of nematode species (marine, free-living, plant-parasitic, and vertebrate and invertebrate parasites) from our taxonomic file revealed 13 of 70 specific descriptions included methods of killing, fixing, or mounting in the original description. All 13 were permanently mounted in glycerine.

Failure to record fixation and preservation methods in descriptions puts a burden on nematologists that make specific determinations. In order to be certain a specimen is a particular species especially when one or more biometric characters are at variance with the original description it would be desirable to approximate the killing and fixing methods of the original describer. This would be almost impossible on the basis of current descriptions. Some of the divergent methods of killing nematodes for taxonomic descriptions include 4 and 5% formalin which in the writer's experience distort and shrink most nematodes severely, or relaxing nematodes with hot water with or without formaldehyde. Maggenti and Viglierchio (1965) stated, "During heat killing the serpentine configuration of a live nematode is altered, its dead body usually changing from a nearly linear to a crescent or acute helix shape; unfortunately characteristic external body configuration may be obtained while internal configuration is completely distorted." They also stated, "Hot water stabilizes organ tissues and allows a wider selection of fixative treatments, but this is also the method's greatest disadvantage. Stabilization by heat causes permanent effects that resist specimen improvement by subsequent chemical treatments."

Lamberti and Sher (1969) compared 10 various preparation methods for killing, fixing, and mounting *Longidorus africanus*. Best results in this study were obtained by killing specimens by Seinhorst's method, fixing in FAA, and mounting in glycerine using the slow method. They did not evaluate lactophenol as a test mountant; however, they stated that they believe lactophenol has a clearing effect on the specimens. They evaluated cold 2.5% formalin as a fixative and stated, "The specimens were badly distorted and twisted and morphological characters were hard to resolve."

Scott (1929) compared lengths of dog hookworms subjected to four fixatives. He found using 20% glycerine in 70% alcohol at 70 C resulted in a length increase of almost 10%.

Stone (1971) found that heating specimens to 65 C followed by fixing in 4% cold formalin causes the smallest dimensional changes. In the author's experience fixing at room temperature in 2% formalin has proved a very satisfactory method for temporary mounts or for long-term storage. Little or no distortion has been noted using this method.

Specimen clearing by lactophenol has not been noted in this study. Specimens mounted in lactophenol for 16 years are still in excellent physical condition in the Division of Plant Industry collection.

Some descriptions (Wasilewska, 1965) are based on measurement of living nematodes. Finally some descriptions are based on glycerine-preserved permanently mounted specimens. Shrinkage due to fixation has been noted numerous times in methodology. An unusual case of shrinkage was reported by Timm (1966) where a 10% shrinkage occurred in specimens remounted in glycerine from 41- to 44-year-old glycerine slide mounts. In the present study shrinkage was demonstrated between 2% cold formalin killed and fixed specimens and the same permanently mounted specimens 6 years later.

In summary, a definite shrinkage in total length was noted which affected all other body measurements. The greatest effect of shrinkage was in the tail and the resultant "gamma" calculation. The effect of time on morphological entities was not pronounced in either method. Obvious structural deterioration was not evident.

It is the writer's opinion based on this study

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that the lactophenol acid fuchsin method may be equated with the glycerine method as a permanent mounting method.

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Tylenchorhynchus kashmirensis sp. n. and *Quinisulcius himalayae* sp. n. (Nematoda: Tylenchorhynchinae) from India

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ABSTRACT: *Tylenchorhynchus kashmirensis* sp. n. is distinctive because of its three labial annules 17- to 22-μ-long, stylet, and subcylindrical tail with 13–17 annules and subhemispherical annulated terminus. *Quinisulcius himalayae* sp. n. is characterized by a hemispherical head with eight annules, 17- to 24-μ-long stylet, areolated lateral field with five incisures, and a tail with 44–58 annules.

The present study pertains to two new species of the subfamily Tylenchorhynchinae Eliava, 1964, collected from India.

Tylenchorhynchus kashmirensis sp. n. (Fig. 1, A–C)

Females (5): L = 0.67 (0.60–0.74) mm; a = 29 (24–34); b = 4.7 (4.0–5.5); c = 32 (24–37); V = 63 (62–64) %; stylet = 19 (17–21) μ.

Holotype female: L = 0.64 mm; a = 29; b = 4; c = 24; V = 63%; stylet = 21 μ.

Allotype male: L = 0.64 mm; a = 23; b = 4.7; c = 14; T = 49%; stylet = 18 μ; spicula = 19 μ.

Position of body on death nearly straight. Lateral field ¼ of body width, made up of four incisures. Longitudinal striations absent. Labial region offset with three annules. Cephalic framework moderately developed. Stylet 17–21 μ long with rounded basal knobs which are laterally inclined. Excretory pore 78–86 μ from anterior end. Hemizonid one to two annules anterior to the excretory pore. Cardia rounded.

Vulva located at 62–64% and protrudes slightly. Intestine with a postanal extension less than one anal body width long. Tail conoid, 1.6 anal body widths long, made up of 13–17 annules and tapering to a bluntly rounded annulated terminus.

Male: Body slightly ventrally arcuate on death. Labial region similar to that of the

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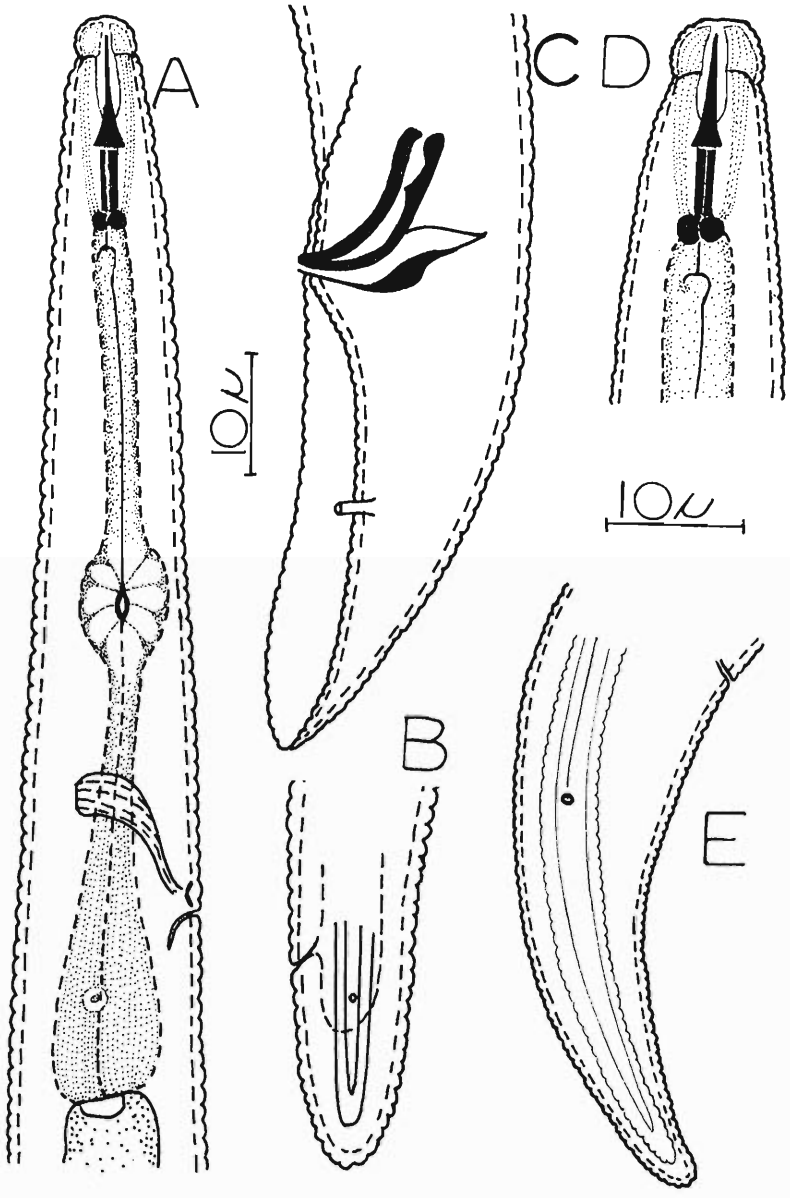


Figure 1, A–C. ***Tylenchorhynchus kashmirensis*** **sp. n. A, Female anterior end. B, Female tail. C, Male tail.**
D–E. ***Quinisulcius himalayae*** **sp. n. D, Female anterior end. E, Female tail.**

female; stylet 17–19 μ long. Excretory pore 84–88 μ from anterior end. Spicules 19–21 μ long. Gubernaculum troughlike, inclined laterally with a median thickening. Tail sharply pointed and completely enveloped by a crenate bursa.

HOLOTYPE FEMALE–ALLOTYPE MALE: Slide Nos. PN/Tylencho/1 and 2, respectively, Nem-

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atode Collection, Post-Graduate Department of Zoology, University of Kashmir, Srinagar, Kashmir, India.

PARATYPES: 5 females and one male distributed as follows: 2 females, slide No. PN/Tylencho/3, same location as above; 3 females and 1 male, National Nematode Collection, Indian Agricultural Research Institute, New Delhi, India.

HOST: From around the roots of a local cultivar (*Hak*) of *Brassica oleracea*.

LOCALITY: Rajbagh, Srinagar, Kashmir, India.

Diagnosis

T. kashmirensis sp. n. differs from other species of the genus by possessing three labial annules, a vulva situated at 62–64%, and a subcylindrical tail with 13–17 annules and an annulated subhemispherical terminus.

It resembles *T. brassicae* Siddiqi, 1961, but can be differentiated by a smaller number of labial annules, distinctive location of the vulva, greater number of tail annules, and different shape of gubernaculum. It also resembles *T. latus* Allen, 1955, and *T. clarus* Allen, 1955, but differs in the number of labial annules, location of vulva, shorter tail, and presence of males.

Genus *Quinisulcius* Siddiqi, 1971

Diagnosis (emended)

Tylenchorhynchinae; body wall curved when relaxed; lateral field with five incisures, sometimes areolated; lip region offset, rounded, finely annulated; cephalic framework weakly sclerotized; female tail conoid ventrally arcuate; males rare, spicules usually with small-sized distal flanges; a large protrusible gubernaculum present with proximal end directed dorsally and with a well-developed bursa.

Quinisculcius himalayae sp. n. (Fig. 1, D–E)

FEMALES (5): L = 0.72 (0.62–0.78) mm; a = 29 (27–31); b = 4 (3.8–4.5); c = 14 (12–16); V = 53 (51–56) %; stylet = 21 (17–24) μ.

HOLOTYPE FEMALE: L = 0.78 mm; a = 31; b = 4; c = 16; V = 56%; stylet = 22 μ.

Position of body on death ventrally arcuate. Lateral field ¼ of body width, areolated, made up of five incisures, the middle one disappearing in the vicinity of the phasmid which is located one anal body width behind the anus. Lip region hemispherical, offset with eight annules. Cephalic framework weakly developed. Stylet 17–24 μ long, basal knobs rounded, sloping slightly posteriorly. Cardia conoid-rounded. Excretory pore located at 120–155 μ from the anterior end. Hemizonid immediately anterior to excretory pore, spreading across four to five annules. Excretory canals well developed and strongly cuticularized. Lateral canals distinct.

Intestine with a postanal extension slightly more than two anal body widths long. Tail three anal body widths long with 44–58 annules and tapering to a conoid striated terminus.

MALE: Not found.

HOLOTYPE FEMALE: Slide No. PN/Quin/I, Nematode Collection, Post-Graduate Department of Zoology, University of Kashmir, Srinagar, Kashmir, India.

PARATYPES: 5 females, distributed as follows: 2 females, slide Nos. PN/Quin/2 and 3, same location as above; 3 females, National Nematode Collection, Indian Agricultural Research Institute, New Dehli, India.

HOST: From around the roots of *Malus sylvestris*.

LOCALITY: Dalhousie, Himachal Pradesh, India.

Diagnosis

These specimens were found in a mixed population that contained *Q. acti* (Hopper, 1959) Siddiqi, 1971, which has been reported by Sethi and Swarup (1968) from adjoining localities. The females of *Q. acti* conformed to the description given by Sethi and Swarup (1968) in the presence of a bluntly rounded lip region with eight annules, a conoid tail with an unannulated, slightly enlarged terminus and a lateral field without areolations.

Q. himalayae sp. n. is distinctive because of its hemispherical head with eight annules, 17–24 μ long stylet, areolated lateral field with five incisures and a conoid striated tail with 44–58 annules. It resembles *Q. acti* (Hopper, 1959) Siddiqi, 1971 but can be differentiated by its longer stylet range, areolated lateral field and annulated tail terminus. It also resembles *Q.*

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goodeyi (Marinari, 1962) Siddiqi, 1971 but differs in the presence of an offset lip region.

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I am grateful to Dr. D. N. Fotedar, Professor of Zoology, University of Kashmir, under whose supervision this work was carried out. This formed part of the thesis accepted by the University of Kashmir for the Ph.D. degree. I am also grateful to Professor A. C. Tarjan, University of Florida, whose suggestions were used in the preparation of this manuscript.

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Paralecithobotrys brisbanensis sp. n. from Australian Mullet (Trematoda: Haploporidae)[1]

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ABSTRACT: *Paralecithobotrys brisbanensis* sp. n. is described from the sea mullet, *Mugil cephalus* L., collected in the Brisbane River, Queensland, Australia. The two other species in the genus have been found in freshwater fish of Africa and South America. It is suggested that freshwater fish probably become infected with such marine trematodes when marine mollusks serving as intermediate hosts become adapted to freshwater.

During sabbatical leave (1970–71) spent in the Parasitology Department, University of Queensland, Brisbane, Australia, haploporid and other trematodes from mullet were studied. Some reported here represent a new species of the genus *Paralecithobotrys* which Teixeira de Freitas (1947) erected with *P. brasiliensis* as type for specimens from freshwater fish, *Lahilliela kneri* Steind. and *Leporinus* sp. in Brazil. The only other member of this genus reported to date is *P. africanus* described by Manter and Pritchard (1964) from an African freshwater fish, *Haplochromis philander* Weber.

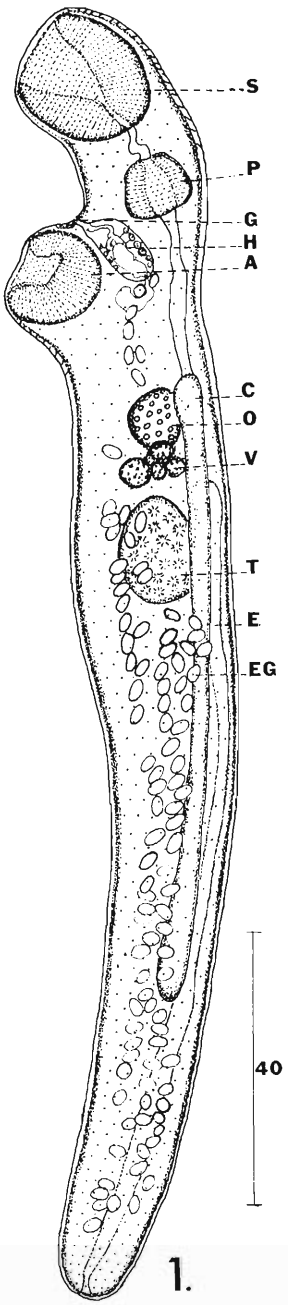
In this study, specimens were fixed without pressure in hot 5% formalin, stained with Mayer's paracarmine, cleared in methyl benzoate, and mounted in Canada balsam. Drawings were made with the aid of a camera lucida

[1] Supported in part by NSF G6962.

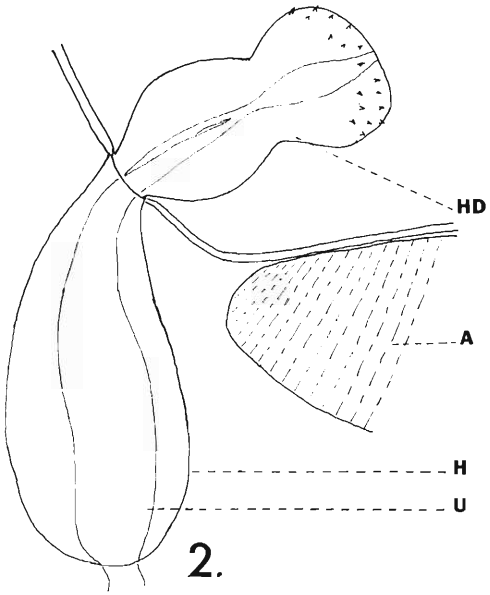
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Figures 1–3. ***Paralecithobotrys brisbanensis*****, lateral view. 1. A, acetabulum; C, cecum; E, excretory vesicle; EG, egg; G, genital pore; H, hermaphroditic sac; O, ovary; P, pharynx; S, oral sucker; T, testis; V, vitellaria. 2. Hermaphroditic duct extruded. HD, hermaphroditic duct; U, uterus. 3. Terminal genitalia, semidiagrammatic. B, prostate bulb; EV, external seminal vesicle; I, internal seminal vesicle.**

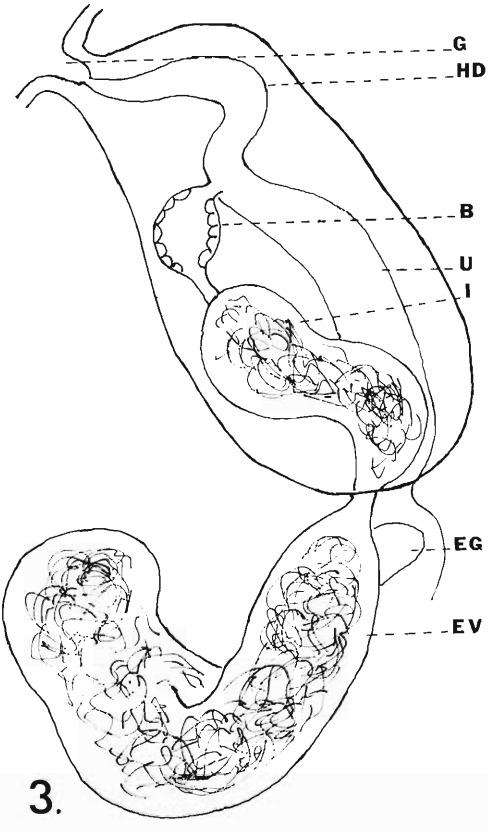
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1.



2.



3.

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unless otherwise stated. Measurements are in microns.

Paralecithobotrys brisbanensis sp. n. (Figs. 1–3)

Diagnosis

With the characters of the genus. Measurements based on three specimens, all viewed from the side because of shape assumed during fixation. Tegument mostly smooth but a few spines dorsoanteriorly and immediately postacetabularly. Neck glands numerous, many with ducts opening anteriorly around oral sucker. Body cylindrical, 1,290–2,506 long, 188–280 wide. Oral sucker terminal to subterminal, 187–218 long, 171–230 wide. Acetabulum in anterior third of body, 134–199 long, 100–152 wide; sucker ratio 1.4:1. Prepharynx 56–68 long. Pharynx 81–106 long, 100–140 wide. Esophagus relatively long and wide, bifurcating at ovarian level; ceca terminate about one-fourth body length from posterior end. Genital pore midventral, immediately preacetabular. Hermaphroditic sac oval, at acetabular level, 146–162 long, 53–75 wide; contains hermaphroditic duct lined with tiny spines or tubercles best seen on everted duct (Fig. 2), distal portion of uterus, prostate cells, prostatic bulb, and internal seminal vesicle. External seminal vesicle a bent tube at acetabular level. Testis oval, single, in anterior half of body, 140–224 long, 96–165 wide. Vitellaria in two lateral clusters of seven to eight follicles each, between levels of testis and ovary. Ovary subspherical, between testis and acetabulum, 90–106 long, 78–109 wide. Uterine seminal receptacle present. Laurer's canal not seen but probably present. Uterus in long loops from near posterior end of body to hermaphroditic pouch. Eggs numerous, shells thin, yellow and operculated, increase in size with growth of embryo, larger ones 37–47 by 22–31 (avg. 43 by 25). Miracidium without eyespots. Excretory vesicle Y-shaped with long stem and short crura reaching to level of vitellaria.

HOST: *Mugil cephalus* L., sea mullet.

HABITAT: Small intestine.

LOCALITY: Brisbane River, Queensland, Australia.

HOLOTYPE: Deposited as No. 7112, Hancock Parasitology Collection, University of Southern California.

Discussion

Paralecithobotrys brisbanensis differs from other species in the genus in having the oral sucker larger than the acetabulum, rather than equal suckers; in the bifurcation of the gut posterior to, rather than at the acetabular level; in the anterior extent of the excretory vesicle; and in having smaller eggs and nonocellate miracidia.

The occurrence of members of this genus in freshwater fish and the sea mullet raises some interesting questions. Is the genus marine or freshwater? The family Haloporidae, to which the genus belongs, contains mostly marine parasites. If the genus is marine, how do freshwater fish become infected? Obviously some mollusk is serving as intermediate host in freshwater. Was this formerly marine or has a new freshwater mollusk been initiated into a haloporid life cycle? Since digenetic trematodes exhibit considerably more host specificity for their molluscan than for their definitive hosts, it seems likely that a marine mollusk has become adapted to freshwater. Such mollusks could have been infected originally by miracidia brought to freshwater by the sea mullet that spends part of its life in the sea and part in freshwater and has a circumglobal distribution. Once the infection is established, new freshwater definitive hosts could be acquired. Some evidence for this type of development has been offered by Martin (1973).

Acknowledgments

I am greatly indebted to Professor J. F. A. Sprent, Head of the Parasitology Department, University of Queensland, Australia, for the use of laboratory facilities and encouragement in many ways; to Dr. John Pearson, Reader in Parasitology, for a great deal of assistance; and to Mr. Jim Davie for help in collecting fish.

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The Chronology of Tapeworm (*Moniezia expansa*) Acquisition by Sheep on Summer Ranges in Montana and Idaho[1]

DAVID E. WORLEY, RICHARD H. JACOBSON, AND RICHARD E. BARRETT[2]

ABSTRACT: Acquisition of *Moniezia expansa* by sheep grazing on summer ranges in the mountains of western Montana and east-central Idaho and on prairie rangeland in eastern Montana followed a distinct seasonal pattern, with 35–72% of the lambs developing patent infections within 6–9 weeks after initial contact with summer grazing areas. The peak prevalence of tapeworms occurred in late August in lambs which had been moved onto summer ranges in early July. A reduced prevalence attributed to spontaneous loss of infections was noted in late September and October. The marked seasonal nature of the infection apparently resulted from mass exposure of lambs to range vegetation contaminated by overwintering oribatid mites containing tapeworm cysticercoids.

The common ruminant tapeworm, *Moniezia expansa* (Rudolphi, 1810), is widely distributed in domestic and wild ungulates, and occurs throughout North America in domestic sheep (Becklund, 1964). In Montana, the number of reported cases of ovine monieziasis increases from a seasonal low in the spring to a peak during the autumn and early winter months (unpublished accession records, Montana State University and the Montana Livestock Sanitary Board). This apparent seasonal periodicity suggests that exposure of lambs to the oribatid mite intermediate host takes place primarily during the late summer and autumn months and is correlated with the use of ranges which are grazed intensively for short periods each year. The purpose of the present study was to determine the cycle of tapeworm acquisition in sheep grazing under these conditions on prairie or mountain rangeland in eastern and western Montana and in east-central Idaho.

Materials and Methods

Acquisition of *Moniezia* by range sheep was monitored in three areas in Montana and Idaho by periodic fecal examinations of lambs and ewes during the months of June through September or October, while the sheep were grazing on summer ranges.

The first study area, the Cache Creek Range, was a subalpine mountain basin located along the eastern slope of the Madison Mountains in Gallatin County, Montana. Approximately 8,000 acres of rangeland consisting of native grasses and forbs interspersed with stands of coniferous forest and aspen groves were used for a 60- to 75-day period each summer by about 1,800 sheep. No other use was made of the area by domestic livestock due to the inaccessibility of the area and the severe weather conditions occurring at higher elevations (7,200–9,400 ft). The second study area, the Big Mountain Range, was similar ecologically, with sagebrush foothills, forested ridges, and open meadows situated along the crest of the Centennial Mountains in Clark County, Idaho, and Beaverhead County, Montana. Approximately 6,000 acres of unfenced summer range were used during July and August by about 2,200 sheep from the U. S. Sheep Experiment Station at Dubois, Idaho. The third area was a fenced shortgrass prairie range in McCone County in eastern Montana. Approximately 3,660 acres of native bunchgrass range were utilized for summer grazing by 2,000 sheep during the months of May through October. The nature of the vegetation and the limited rainfall in this region restricted its use for sheep pasture to about 6 months each year. No other use was made of this range by domestic animals.

A modified Lane centrifugal flotation technique (Dewhirst and Hansen, 1961) was used to detect cestode ova in fecal samples collected directly from sheep or from samples deposited on the ground. Prevalence of the infection was estimated by periodic fecal examinations of approximately 5–10% of the lambs in each of the three groups under investigation. Ages of the lambs varied from 2 to 6 months during

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the observation period. Intensity data were compiled from worms recovered from older lambs (6–8 months of age) which were necropsied in November and December of 1964–65. Individual worm burdens were determined by counting the number of scolices present in the small intestine at necropsy. The total length of strobila in each animal was calculated from worms taken directly from the host, relaxed in tap water, and measured in a normally extended position in a thin film of water.

Each of the three study areas had a history of use as sheep range, and had been used exclusively for this purpose for at least 10 years prior to this study. Data from the Cache Creek area were collected over the 4-year period from 1964 through 1967. The Centennial range study was done in 1967–68, and observations were made in eastern Montana in 1968.

Results

Seasonal prevalence

Acquisition of *M. expansa* occurred primarily in July and August during each year of the study at each of the three locations. In all instances, groups of lambs which were entirely or virtually free of patent infections in late June or early July were 35–61% positive for *Moniezia* by late August. In 1964, the prevalence of tapeworms in lambs grazing the Cache Creek range increased from 0 to 55.5% between 1 July and 30 September. In 1965, a similar trend was noted, with the peak prevalence (41.3%) occurring in late August. By late September, the proportion of infected lambs had decreased to 34%. The following summer, the peak of the tapeworm season again occurred in August but persisted through September. In 1967, a maximum prevalence of 61% was reached in late August in lambs which had been grazing on summer ranges for approximately 2 months. After 3 months on range, 50% of the lambs still were positive for tapeworms. Prevalence of *Moniezia* in adult ewes grazing with these lambs varied between 10 and 16%.

A similar rate of tapeworm acquisition was observed in lambs grazing in the Centennial Mountains on the Idaho–Montana border. During two successive years, the proportion of lambs positive for *Moniezia* increased from 0 to 71% and 45%, respectively, after 8 weeks on summer ranges in July and August. In eastern Montana, the proportion of infected lambs increased from 0 to 72.8% during the period from June to mid-July 1968. The prevalence of tapeworms in this band declined to 46.5% during the following 8-week period, after which the lambs were unavailable for further examination. At the seasonal peak of the infection in the lambs, 10% of the ewes were positive for *M. expansa*.

Intensity of infections

The average number of tapeworms was three in a series of 23 lambs necropsied in November and December of 1964–65. Individual strobilae averaged 112.01 cm in length, and varied from 12.7 to 594.3 cm. The length of the largest specimen recovered from a lamb in this study exceeded the maximum strobilar length listed for *M. expansa* by both Stiles and Hassall (1893) and Spasskii (1951).

Discussion

The seasonal development of monieziasis noted in lambs during this study apparently resulted from exposure of sheep to oribatid mites harboring tapeworm cysticercoids acquired during previous grazing seasons. Because use of the Cache Creek and Big Mountain ranges by sheep was restricted to a 60- to 80-day period each year between July and mid-September, and reuse of previously grazed areas was not permitted until the following year, infections presumably originated from residual contamination of range vegetation. Both Stoll (1935) and Hawkins (1948) observed a similar persistence of pasture infectivity in New Jersey and Michigan which they attributed to residual populations of infected mites on pastures. In Russia, Potemkina (1959) found that oribatids infected with cysticercoids of *M. expansa* survived for 24 months. In the present study, the appearance of a "wave" of patent infections in lambs 6 to 9 weeks after initial contact with summer ranges also indicates that overwintering tapeworm larvae in mites were responsible for the distinct seasonal nature of the cycle. A similar correlation between overwinter survival of infected mites and the seasonal onset of monieziasis in lambs was observed by Prokopic (1967) in Czechoslovakia.

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The minimum time required for development of the cysticercoid stage of *M. expansa* in oribatids is 16 weeks (Stunkard, 1938). Under field conditions, 4 to 5 months often are required before the cysticercoid is infective (Prokopic, op. cit.). Thus, the possibility that fully developed cysticercoids were available to sheep in the present study during the same grazing season in which infection of the mites occurred was eliminated. Movement of the sheep on a predetermined circuit through the available grazing areas prevented contact with vegetation contaminated by infected ewes during the same grazing season in both the Cache Creek and Big Mountain range bands. In eastern Montana, patent infections appeared in a majority of the lambs before mature cysticercoids would have been present in oribatids infected during the same grazing season.

The prepatent period of *M. expansa* in sheep averages 25–35 days (Hawkins, 1948) or 40–41 days (Al'kov, 1971), which indicates that the maximum exposure of sheep to infected oribatids occurred about the 2nd or 3rd week in July on the Cache Creek range. The area in which the sheep were grazing each year at that time was a series of mountain meadows in open stands of spruce and fir at elevations varying from 8,000 to 9,400 ft. Factors contributing to a high intake of mites in this portion of the range may be related to the relatively sparse herbage just below timberline which necessitated closer and more intensive grazing in order to support the requirements of the sheep.

An additional source of range contamination with *Moniezia* ova which theoretically could contribute to the high rate of infection noted in sheep in this study exists in populations of wild ruminants present in each of the areas studied. Mule deer, which are present in all three areas throughout the year, are susceptible to *Moniezia* spp. (Honess and Winter, 1956), as are pronghorn antelope, which were relatively common in area three. Infections with *Moniezia* sp. also occur occasionally in elk (Worley et al., 1969), which inhabit both the Cache Creek and Big Mountain ranges. It seems improbable, however, that the volume of tapeworm ova passed by a limited number of wild ruminants could contribute significantly to environmental contamination on ranges used intensively by much larger numbers of sheep.

The rapid acquisition of *M. expansa* by lambs at all three locations studied suggests that near-optimum conditions existed for exposure on summer ranges used for a limited time between July and mid-September each year. The clinical significance of tapeworm infections in sheep maintained under the conditions of this study is not known. However, there was no evidence of an acute form of monieziasis such as that observed by Lafenetre in France (Euzeby, 1967) or in Russia by Skrjabin and Schul'ts (1934). Spontaneous loss of infections during the post-range period and shipment of lambs to other locations for feeding would tend to obscure any more subtle effects of monieziasis such as borderline anemia and depressed hemoglobin and hematocrit levels which were noted by Hansen, Kelley, and Todd (1950) in lambs with pure *M. expansa* infections. It is possible that worm burdens such as these noted in the present study would be completely asymptomatic, as observed in naturally infected lambs by Hawkins (1946) and in experimentally infected sheep by Kates and Goldberg (1951).

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Paracardicoloides yamagutii gen. et sp. n. from an Australian Eel (Trematoda: Sanguinicolidae)[1]

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ABSTRACT: *Paracardicoloides yamagutii* gen. et sp. n. is described from the blood vessels of Australian eels, *Anguilla reinhardtii* Steindecker, collected in the Brisbane River and one of its tributaries, Moggill Creek, near Brisbane. It differs from other fish blood flukes with two testes in having a raised oral disc, spines sheathed in tegument, an esophageal bulb, and testes close together in the hindbody.

During a sabbatical (1970–71) spent in the Department of Parasitology, University of Queensland, Brisbane, Australia, two small eels, *Anguilla reinhardtii* Steindecker, were collected, one in the Brisbane River and one in Moggill Creek, a tributary of the Brisbane River, and were found to harbor blood flukes. These worms are described as a new genus and species.

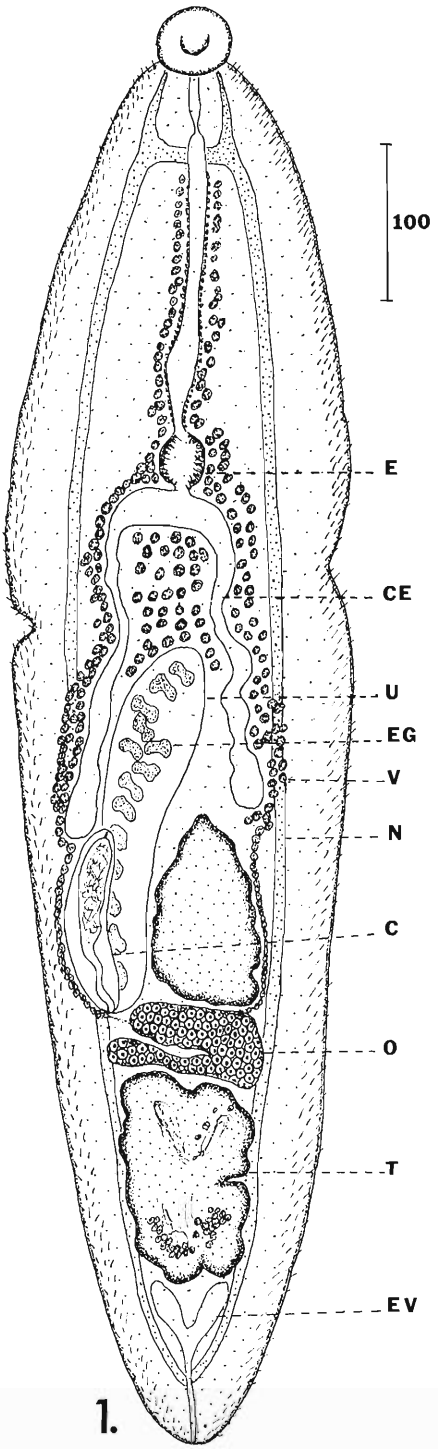
They were fixed without pressure in hot 5%

[1] Supported in part by NSF G6962.

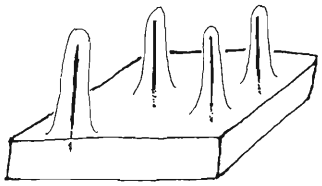
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Figures 1–4. ***Paracardicoloides yamagutii*** **gen. et sp. n. 1. Ventral view. C, cirrus pouch; CE, cecum; E, esophageal bulb; EG, egg; EV, excretory vesicle; N, nerve cord; O, ovary; T, testis; U, uterus; V, vitellaria. Size scale applies to Fig. 1. 2. Diagram of portion of tegument to show sheathed spines. 3. Side view of anterior end showing pedunculate oral disc. 4. Terminal genitalia. G, genital atrium; P, prostate cells; S, seminal vesicle.**

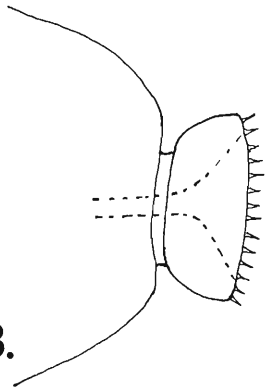
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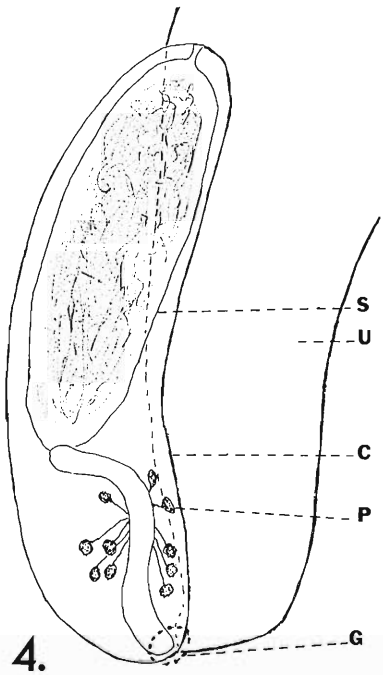
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formalin, cleared in methyl benzoate, and mounted in Canada balsam. Measurements are in microns, averages in parentheses.

Paracardicoloides gen. n.

GENERIC DIAGNOSIS: Small, lanceolate body, dorsoventrally flattened, partially spined. Oral disc raised from rest of body. Esophagus with bulb near gut bifurcation. Posterior ceca only. Vitellaria lateral to most of digestive tract but also intercecal anterior to uterus. Gonads in tandem in hindbody, ovary between two testes. Uterus with swollen, sausage-shaped portion. Cirrus pouch well developed, containing seminal vesicle, a few prostate cells, and a narrow cirrus. Common genital pore on dorsal side. Excretory bladder Y-shaped. Type species: *P. yamagutii* sp. n.

Paracardicoloides yamagutii sp. n. (Figs. 1–4).

SPECIFIC DIAGNOSIS: With characters of genus. Body with ventrolateral band of spines from near anterior to near posterior end on each side. Spines randomly arranged with each spine sheathed in tegument, spines about 6 long. Spinelike projections on oral disc seem to be tegument only. Measurements based on four specimens.

Body 518–924 (704) long and 84–238 (173) wide. Oral disc diameter 28–44 (37). Esophagus 196–264 (238) long, glandular externally along most of its length, with conspicuous posterior bulb having thick wall and cellular extensions into lumen. Ceca narrow, extend posteriorly 90–236 (153). Esophageal bulb and ceca may contain host's blood pigment. Ovary deeply indented, 30–50 (44) anteroposteriorly, 16–109 (70) transversely. Vitellaria from ends of ceca to near level of cerebral commissure; vitellarian ducts pass posteriorly from near ends of ceca to ovarian level. Mehlis' gland poorly developed. Uterus slightly coiled, passes anteriad to empty into expanded portion which contains most of the eggs. Expanded part of uterus 149–224 (187) long, 31–63 (47) wide. Eggs with delicate shells that were badly collapsed during fixation. Posterior end of uterus narrows and empties into genital atrium. Testes with scalloped margins, 31–152 (92) long, 31–115 (81) wide. Vasa efferentia unite anterior to anterior testis to form a relatively short vas deferens which empties into anterior end of cirrus pouch. Cirrus pouch 81–102 (92) long, 28–31 (30) wide. Nerve cords and commissure conspicuous. Arms of excretory bladder extend to near posterior margin of posterior testis.

HOST: *Anguilla reinhardtii* Steindecker, 1867.

HABITAT: Blood vessels, dorsal aorta.

LOCALITY: Brisbane River and tributaries, Queensland, Australia.

HOLOTYPE: Deposited as No. 7113, Hancock Parasitology Collection, University of Southern California.

Discussion

Two other genera of fish blood flukes with two testes, *Paracardicola* Martin, 1960, and *Neoparacardicola* Yamaguti, 1970, have been described. Yamaguti (1970) placed these in a new subfamily, Paracardicolinae. *Paracardicola hawaiensis* Martin, 1960, was recovered from the blood vessels of a puffer fish, *Tetraodon hispidus* L., and *Neoparacardicola nasonis* Yamaguti, 1970, from coelomic washings (presumably from blood vessels) of *Naso hexacanthus* (Bleeker). Both species of fish were collected in Hawaii.

The above fluke genera have either an H- or X-shaped intestine but in *Paracardicoloides* it is an inverted U. Only *Paracardicoloides* has an esophageal bulb, a pedunculate oral disc, spines covered with tegumentary sheaths, and testes close together. The esophageal bulb is similar to that of *Orchispirium heterovitellatum* Madhavi and Rao, 1970, but otherwise they differ greatly.

Two of the four worms described here were not ovigerous. The smallest was from an eel only 21 cm long. It seems likely that eels become infected with this fluke in freshwater. Two species of sanguinicolid cercariae were found in the snail, *Posticobia brazieri* (Smith), collected in the same waters as the eels. Probably one of these is the larva of *Paracardicoloides yamagutii* but time did not permit work on the life cycle.

Live worms were capable of swimming in physiological saline by undulations of the body

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in the dorsoventral plane and wavelike movements of the body margins.

Acknowledgments

I am greatly indebted to Professor J. F. A. Sprent, Head of the Parasitology Department, University of Queensland, for the use of laboratory facilities and encouragement in many ways; and to Dr. John Pearson, Reader in Parasitology, for a great deal of assistance.

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Helminth Parasites of the Common Eider Duck, *Somateria mollissima* (L.), in Newfoundland and Labrador[1]

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ABSTRACT: One hundred and ten common eider ducks, *Somateria mollissima* (L.), were obtained from six sampling stations around the Newfoundland coast during the period November 1968–July 1969. The birds were examined for metazoan parasites using conventional parasitological techniques. Blood smears were made when possible. Twenty-one genera of parasites (7 trematodes; 3 cestodes; 7 nematodes; 1 acanthocephalan; 1 haematozoan; 1 mallophagan; 1 siphonapteran) were recovered. Six new host records, and 12 new records for common eiders in North America are noted. Ninety-five per cent of the ducks were infected with helminth parasites, the number of species per infected bird ranging from 1 to 13 (mean 8). Details of infections with the various species are given, each species being discussed and/or described individually. Only two species of helminths (*Echinuria uncinata* and *Polymorphus botulus*) caused observable damage, and none were lethal.

The common eider duck [*Somateria mollissima* (L.)] has a Holarctic distribution (Godfrey, 1966) and is the commonest marine duck in Newfoundland waters, being particularly abundant in the winter months, when it inhabits the littoral and sublittoral zones in large flocks. Many aspects of the biology of the common eider have been studied, including its parasites, due to its economic importance, throughout the whole of its range. In North America some of the more recent works on the helminthofauna of common eiders are those of Clark et al. (1958), Schiller (1955), Stunkard (1960a, b, 1964, 1966, 1967a, b), Stunkard and Uzmann (1958), and van Cleave and Rausch (1951), while in the USSR Belopolskaya (1952), Kulachkova (1953, 1954, 1957, 1958, 1960), and Ryzhikov (1960, 1963a, b, c, 1965) worked on this host.

A study was therefore initiated to determine the nature of the helminth burden of common eiders in Newfoundland. This work constitutes the first such survey of this host from the eastern seaboard of North America.

Materials and Methods

Common eiders were collected, during the period November 1968–July 1969, at six localities along the east coast of insular Newfoundland and southern Labrador [1. Witless Bay 47° 15′ N, 52° 50′ W); 2. Duck Island, near Herring Neck, Notre Dame Bay (49° 35′ N, 54° 35′ W); 3. near Long Island, N.D.B. (49° 30′ N, 55° 35′ W); 4. Grey Islands (50°47′ N, 55° 35′ W); 5. Hare Bay (51° 15′ N, 55° 45′ W); 6. St. Peter Bay (52° 02′ N, 55° 55′ W)]. Adult birds were shot, using a 12-gauge shotgun and No. 2 shot, while chicks were normally caught in a large dip net. All specimens were

[1] This paper consists largely of material submitted by the senior author in partial fulfillment of the requirements for the degree of M.Sc., Memorial University.

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Table 1. Details of infection of common eider ducks [*Somateria mollissima* (L.)] with trematodes.

Parasite	Per cent infection	Range No./infected bird	Mean No./infected bird	Status
Himasthla compacta Stunkard, 1960	16	1–145	32	*
Cryptocotyle lingua (Creplin, 1825)	3	1–3	2	**
Microphallus primas (Jagerskiöld, 1908)	5	1–492	107	**
Microphallus pygmaeum (Levinsen, 1881)	82	1–67,140	6,377	***
Maritrema subdolum (Jagerskiöld, 1908)	2	1	1	**
Gymnophallus bursicola (Odhner, 1900)	60	1–635	85	***
Gymnophallus choledocus (Odhner, 1900)	47	1–12	4	***
Gymnophallus minor (Ryzhikov, 1965)	69	1–3,035	173	**
Renicola sp.	31	1–550	76	**
Notocotylus attenuatus (Rudolphi, 1809)	79	1–261	13	**

Status: * new host record; ** new records for common eiders in North America; *** new records for Newfoundland.

Table 2. Details of infection of common eider ducks [*Somateria mollissima* (L.)] with cestodes, nematodes (adults), and Acanthocephala.

Parasite	Per cent infection	Range No./infected bird	Mean No./infected bird	Status
Lateriporus teres (Krabbe, 1869)	12	1–45	13	–
†*Hymenolepis* (*Microsomacanthus*) sp.	85	1–23,084	2,547	–
Fimbriarioides intermedia (Fuhrmann, 1913)	37	1–189	37	***
Capillaria nyrocinarum (Madsen, 1945)	47	1–44	6	**
Amidostomum acutum (Lundahl, 1848)	44	1–80	6	**
Streptocara crassicauda (Creplin, 1892)	11	1–4	2	**
Echinuria borealis (Mawson, 1956)	3	1–4	3	*
Echinuria uncinata (Rudolphi, 1819)	1	97	97	**
Paracuaria tridentata (Linstow, 1877)	3	1	1	*
Tetrameres somateriae (Ryzhikov, 1963)	40	1–39	5	**
Polymorphus botulus (Van Cleave, 1916)	92	1–654	80	–

Status: * new host record; ** new records for common eiders in North America; *** new records for Newfoundland.

† 3 species [*H.* (*M.*) *formosoides**; *H.* (*M.*) *microskrjabini**; *H.* (*M.*) *somateriae***] discussed individually in text.

weighed, measured, sexed, and the skin retained as part of a wider study.

Most of the specimens collected in the winter were deep-frozen prior to examination. When freezing facilities were not available the birds were eviscerated in the field, the viscera then being preserved in 10% formalin for later examination.

The ducks were examined for ectoparasites and endoparasites using conventional techniques. The digestive tract was divided into number of sections, namely the esophagus, proventriculus, gizzard, duodenum, small intestine (further subdivided into four equal sections), large intestine, ceca, and cloaca, to determine the linear distribution of any parasites found.

Ten nests were collected in 1969 from areas 5 and 6. Down and plant material, sealed in plastic bags, was taken to the laboratory and left in a warm place to facilitate hatching of parasite eggs or emergence of adults from any pupae present.

Parasites that were recovered were fixed and stored in 5% formalin or 70% alcohol. At a later date, when necessary for identification, specimens were stained [Semichon's acetic carmine for trematodes, cestodes, acanthocephalans; Grenacher's borax carmine, cestodes, acanthocephalans; Celestine blue (*Celestin*, Riser, 1949), cestodes], dehydrated, cleared, and mounted. Nematodes and ectoparasites were mounted and cleared in Rubin's fluid, while blood smears were air-dried, fixed in 100% ethanol, and stained with Giemsa.

Results and Discussion

One hundred and ten common eiders (61 females, 49 males; chicks and adults) were examined during the survey, a total of 21 genera of parasites being recovered (7 trematodes, 3 cestodes, 7 nematodes, 1 acanthocephalan, 1 haematozoan, 1 mallophagan, 1 siphonapteran). One hundred and four birds (95%) were found to be infected with helminths (Tables 1, 2), the number of species per infected bird ranging from 1–13 (mean 8). All measurements are given in *microns* unless otherwise stated.

Trematoda

Ten species of Digenea, belonging to seven genera (Table 1), were identified, the number of species per bird ranging from 1–8 (mean 4).

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Himasthla compacta was first described (Stunkard, 1960b) from laboratory-reared herring gulls (*Larus argentatus* Pont.), which had been fed soft-shelled clams (*Mya arenaria* L.) containing echinostome cercariae, while attempts to infect laboratory-reared eider ducks failed.

Measurements of the present specimens, the majority of which were found in the duodenum and first quarter of the small intestine, agreed with those of Stunkard (1960b) with a few exceptions (body length, lineal spines, testes slightly smaller). The minor variations noted may have been due to differences in technique, state of maturity (most immature), or host influence.

Only birds collected in Hare Bay (Area 5) contained this helminth. Differences in the feeding habits of the birds or in the presence of suitable intermediate hosts in Hare Bay might explain this anomalous distribution.

Seven specimens of *Cryptocotyle lingua* were recovered from the duodenum and small intestine of three birds. Infection of the final host is through ingestion of a suitable fish intermediate host (Stunkard, 1930). Although fish is an uncommon item in the eider's diet (Cottam, 1939; Pretsov and Flint, 1963), fish scales, vertebrae, muscles, and eggs were noted in the gut contents of a number of birds during the present study.

Two species belonging to the genus *Microphallus* (Ward, 1901) were recovered, namely *M. primas* and *M. pygmaenum*. Five birds were infected with *M. primas*, the majority of the helminths being located in the posterior half of the small intestine. Smaller numbers of this helminth were seen in the anterior half of the small intestine, large intestine, and ceca. By far the most abundant digenean was *M. pygmaenum*, the number per infected bird ranging from 1–67,140 (mean 6,377). In light infections this species was normally found in the posterior half of the small intestine, while in heavy infections specimens tended to be more widely distributed along the length of the gut (posterior to the gizzard). Kulachkova (1958) noted this helminth in common eiders (78.6% adults, 84.5% chicks infected) from the Kandalaksha Gulf, the average intensity of infection of adult birds being 15,433 (chicks, 92,000) with a maximum intensity of 135,870 (chicks, 640,000). The present results differ somewhat from those of Kulachkova (1958), in that chicks were the most lightly infected birds, while juveniles were the most heavily infected.

Two common eiders from Hare Bay (Area 5) were infected (1 helminth/bird) with *Maritrema subdolum*.

The classification of the genus *Gymnophallus* Odhner, 1900, is at the present time somewhat confused, the validity of many species being somewhat questionable (Stunkard and Uzmann, 1958). During the present study, using existing descriptions of species (James, 1964; Odhner, 1900; Stunkard and Uzmann, 1958) three adult forms were identified, namely *G. bursicola*, *G. choledochus*, and *G. minor*. *G. bursicola* was recovered from the bursa Fabricii and cloaca of 60% of the birds examined. No chicks were infected and while the total number of birds in each age category infected was quite uniform, in the older birds (in which the bursa Fabricii is smaller) the intensity of infection was lighter.

G. choledochus was noted in the gall bladder of 52 (47%) of the birds examined, the greatest intensity of infection being seen in juveniles.

G. minor showed a distinct predilection for the posterior quarter of the small intestine, smaller numbers being found in the large intestine, ceca, and anterior regions of the small intestine. Several species of *Gymnophallus* have been described from the small intestine of common eiders, including *G. minor*, *G. skrjabini* Ryzhikov, 1963, and *G. somateriae* (Levinsen, 1881). All the aforesaid are very similar in appearance and size and it may well be that they are not true species but merely variants of a single species. Stunkard and Uzmann (1958), in commenting on the confusing situation with regard to the status of species within the genus *Gymnophallus*, posed several questions, one of which concerned the validity of classifying helminths from the various parts of the digestive tract of common eiders as distinct species. They state (p. 300), "The extent of morphological variation that may result from development in different hosts or different locations is quite unknown."

To date two species of *Renicola* Cohn, 1904, have been described from common eider ducks, namely *R. somateriae* Belopolskaya, 1952, and

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Table 3. Measurements* (mean (range)) of *H. (M.) formosoides*, *H. (M.) microskrjabini*, and *H. (M.) somateriae* obtained in the present study compared with those of previous workers.

Morphological criteria	*H. (M.) formosoides*		*H. (M.) microskrjabini*			*H. (M.) somateriae*	
	Present study	Tolkacheva, 1966	Tolkacheva, 1966	Present study	Denny, 1969	Present study	Ryzhikov, 1965
Length (mm)	3.0 (2.2–4.0)	1.4	2.0	6.2 (2.5–9.2)	2.5–4.5	9.7 (7.9–12.0)	5.8–7.2
Width (neck)	94 (60–125)	90†	42 × 11	116 (98–228)	109–117	139 (106–161)	–
Width (max)	332 (266–404)	23†	50†	436 (286–542)	475–515	267 (204–386)	230
Scolex, width	257 (204–302)	140–230	230	221 (185–256)	197–250	262 (190–415)	230
length	130 (108–139)			120 (115–112)	92–109	128 (110–149)	128
Suckers, width	111 (96–134)	70–120	80–110	99 (91–105)	54 –65	113 (77–168)	80
Rostellar hooks, length	35 (35)	32– 34	44	42 (40– 44)	39– 43	60 (58– 61)	53– 56
length	24 (18– 30)	23– 42	27– 32	33 (21– 42)	36– 54	33 (23– 49)	
Testes, width	19 (12– 24)	29– 46	23– 27	26 (18– 38)	25– 32	27 (20– 48)	15– 18
length	130 (103–150)	130–140	160–170	173 (144–204)	219–230	114 (85–156)	76–116
Cirrus pouch, width	42 (37– 46)	30	21	22 (18– 25)	–	21 (17– 25)	20
Ovary	–	90	–	–	–	–	–
length	41 (27– 57)	46– 56	–	24 (16– 37)	–	20 (13– 32)	
Seminal receptacle, width	15 (10– 24)	42– 46	–	16 (8– 24)	–	15 (10– 23)	15– 18
length	36 (23– 50)	30– 40	40	44 (30– 67)		29 (16– 45)	
External sem. vesicle, width	23 (14– 36)	30	50	30 (20– 45)	50– 69	21 (10– 31)	18– 20
length	19 (18– 20)	17– 23					
Eggs, width	16 (15– 18)	15– 25	–	35 (32– 40)	33– 38	–	–

* In microns, unless otherwise stated.
† Validity of these figures doubtful, do not agree with dimensions of illustration.

R. mollissima Kulachkova, 1957. During the present study specimens of a *Renicola* sp. were located in the renal tubules of 35 birds, the heaviest infections being seen in juveniles. No chicks were infected.

Notocotylus attenuatus was recorded from the common eider in North America for the first time. This helminth was generally located in the posterior quarter of the small intestine, large intestine, cloaca, and ceca. The latter region was the most heavily infected. Measurements of the present specimens agreed closely with those of Dubois (1951). The only difference was the presence of two to 13 previtelline loops, as opposed to the two to five noted by the above worker.

Dubois (1951) revised the genus *Notocotylus* Diesing, 1839, synonymized *N. attenuatus* (Rudolphi, 1809) with *N. triserialis* Diesing, 1839, and presented evidence to show that *triserialis* should be considered the genotype. Later workers (Yamaguti, 1958; *vide* McDonald, 1969) do not agree with this change and retain the name *N. attenuatus*, as was done during the present work.

Cestoda

Five species of cestodes, belonging to three genera (Table 2), were identified.

Lateriporus teres (13 birds infected) and *Fimbriarioides intermedia* (41 birds infected) are both common parasites of eider ducks. The former species was generally located in the posterior quarter of the small intestine, with lesser numbers appearing elsewhere in this organ, while the latter was most common in the duodenum.

The digestive tract of 94 birds was found to contain members of the genus *Hymenolepis* Weinland, 1858, in numbers ranging from 1–23,084 (mean 2,547). Distribution within the gut was as follows: duodenum, 38.4% of the total helminth burden; small intestine, region 1, 42.5; region 2, 16.3; region 3, 2.2; region 4, <0.5; gizzard, large intestine, and ceca, each <0.5.

Using hook characters as a preliminary aid to identification, an attempt was made to determine the number of species present and the relative abundance of each species in the various regions of infected birds. A sample of scolices (50 when available) was taken from

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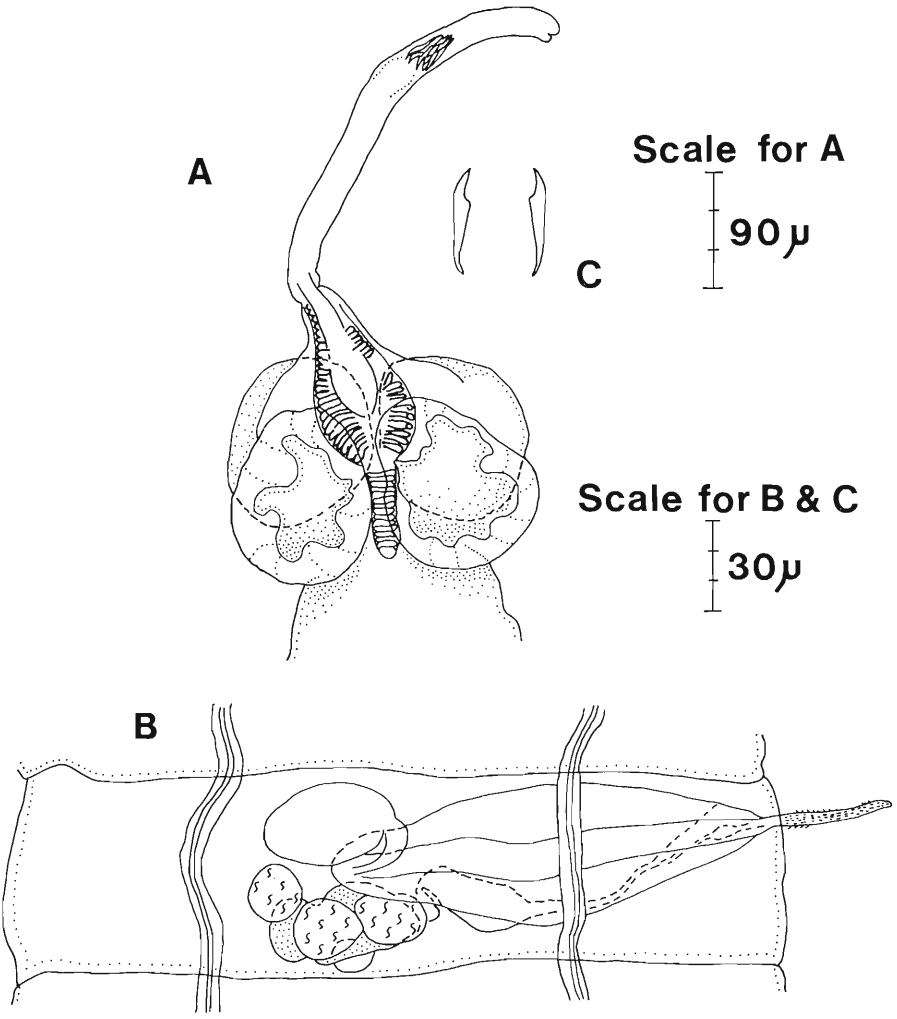


Figure 1. ***Hymenolepis* (*Microsomacanthus*) *formosoides*: A, Scolex with everted rostellum; B, mature proglottid; C, rostellar hooks.**

each section of the gut of 24 heavily infected birds. The size and shape of the hooks from 2,797 scolices were noted (mean 117 per bird), this revealing hooks ranging in size from 31–66. Three groups of hook sizes emerged with peaks at 35–36, 43–48, and 57–62. Intermediate measurements at 39–40 and 51–52 were noted, these being the overlapping ends of the major group ranges. These figures indicated that three species were present, subsequent identification showing them to be *Hymenolepis* (*Microsomacanthus*) *formosoides*, *H.* (*M.*) *microskrjabini*, and *H.* (*M.*) *somateriae*. In order to determine whether or not any other species were present a further series of scolices, from the other 70 infected birds, were examined (10 per gut section, when available). No other species were seen.

The measurements of the present specimens agree closely (Table 3, Figs. 1–3) with those of previous workers. The slight discrepancies may be due to differences in the state of mat-

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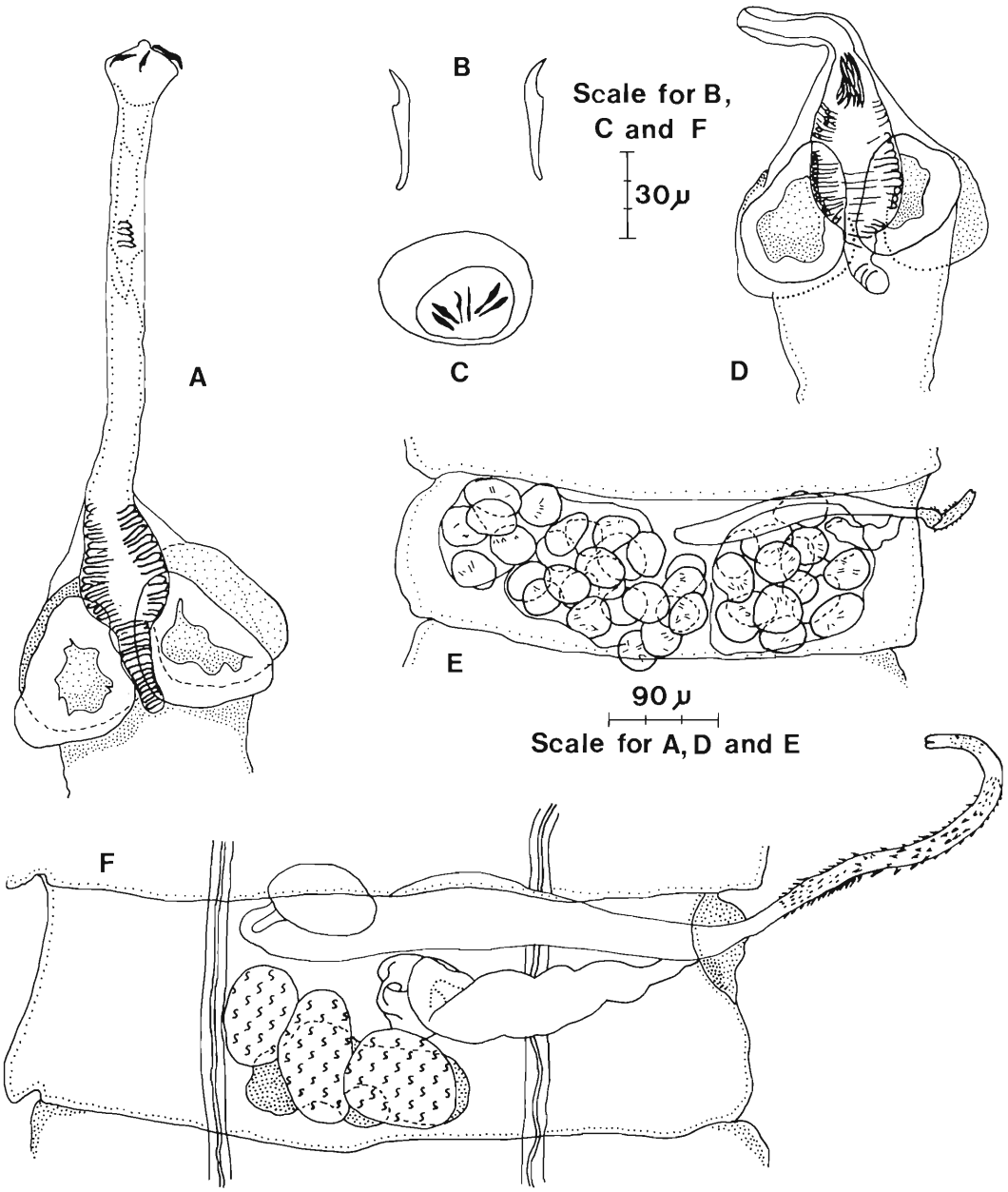


Figure 2. *Hymenolepis* (*Microsomacanthus*) *microskrjabini*: A, scolex with everted rostellum; B, rostellar hooks; C, egg; D, scolex with inverted rostellum; E, gravid proglottid; F, mature proglottid.

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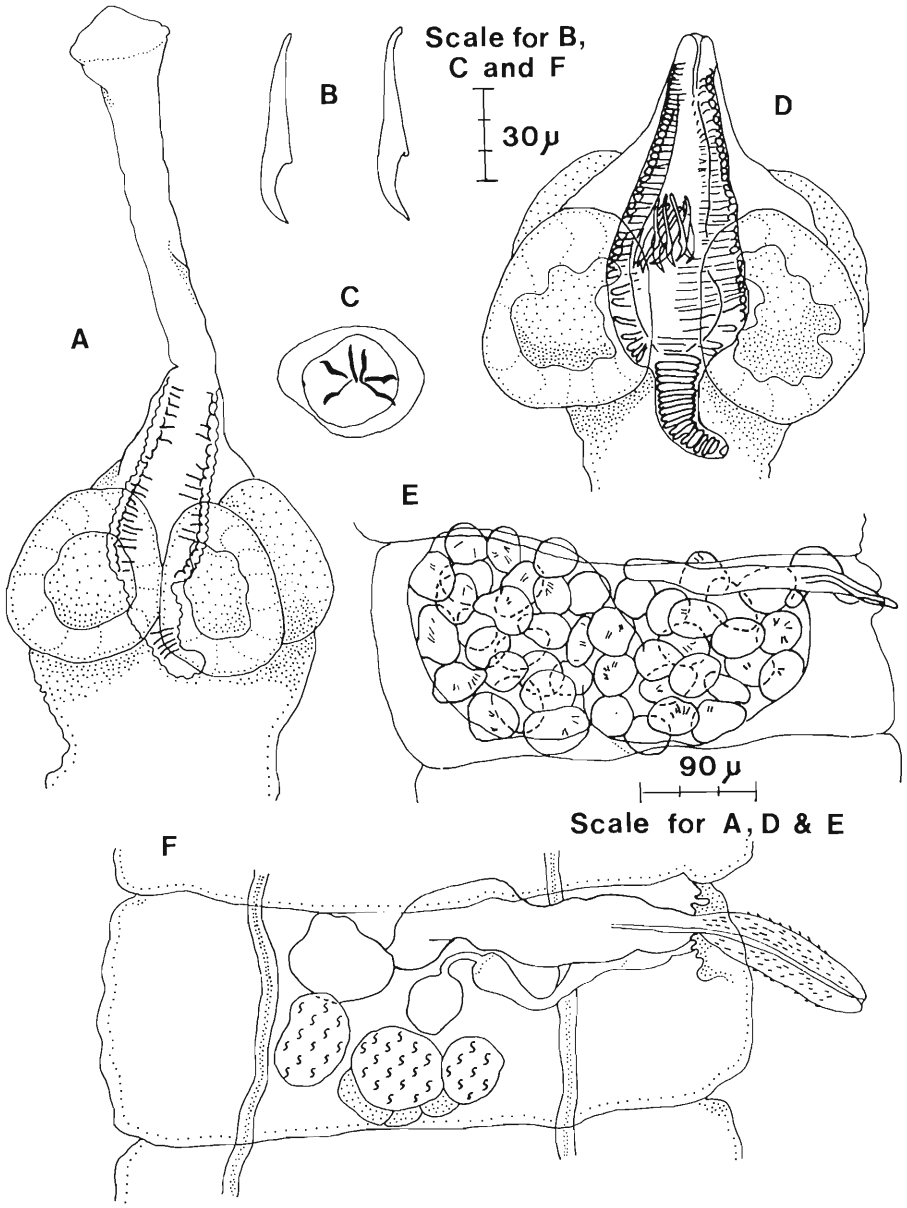


Figure 3. ***Hymenolepis*** **(*Microsomacanthus*)** ***somateriae*****: A, scolex with everted rostellum; B, rostellar hooks; C, egg; D, scolex with inverted rostellum; E, gravid proglottid; F, mature proglottid.**

urity of the helminths, differences in techniques of preparation and measurement, and different host influence. Fourteen species of *Hymenolepis* (*Microsomacanthus*) have been described from the common eider to date (*vide* McDonald, 1969), including five from North America (Schiller, 1955). In a genus as large as this it is not unreasonable to expect slight differ-

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ences in the morphological criteria of a single helminth species in a single host species, particularly when the range of the latter is large and a number of subspecies are known.

Nematoda

Seven species of adult parasitic nematodes, three larval forms, and seven types of free-living larvae and adults were noted during the study. A total of 80 (73%) birds were infected with parasitic forms.

The two most commonly found adult parasitic forms were *Capillaria nyrocinarum* (gizzard to cloaca, oviducts) and *Amidostomum acutum* (below gizzard lining, especially at junction with proventriculus and duodenum). Madsen (1945) described *C. nyrocinarum* from eight species of diving ducks in Denmark, including the common eider. No chicks were infected with this species. Czaplinski (1962) revised the genus *Amidostomum* Railliet and Henry, 1909, reducing the number of valid species from 17 to 6. Kulachkova (1958) recorded *A. boschadis* from common eiders for the first time in the USSR, this being a species that Czaplinski (1962) relegated into synonymy with *A. acutum*, the latter name having precedence.

Streptocara crassicauda, a characteristic helminth of waterfowl (*vide* McDonald, 1969), was seen in the proventriculus and gizzard of 12 birds. Measurements of the present specimens agreed with those given by Gibson (1968) in his revision of this genus.

Two species of *Echinuria* Soloviev, 1912, namely *E. borealis* and *E. uncinata*, were recovered in small numbers. The former species was described by Mawson (1956) from king eiders [*Somateria spectabilis* (L.)] and old-squaw [*Clangula hyemalis* (L.)] taken in the Hudson Bay region of Canada. In the case of the latter species the helminths were located in four nodules, two in the posterior part of the proventriculus (44 worms, 18 females, 26 males) and two in the anterior region of the gizzard (53 worms, 32 females, 21 males).

Paracuaria tridentata, a nematode characteristic of lariform birds (*vide* McDonald, 1969) is reported from common eiders for the first time.

Forty per cent of the birds examined were infected with *Tetrameres somateriae* which was described from a series of males taken from common eiders in the USSR by Ryzhikov (1963c). All the specimens obtained in the present study were males, whose measurements agreed with those given by Ryzhikov (1963c) with the exception of the distance between the anus and the posterior end [present study 191 (160–223): Ryzhikov 245 (224–261)].

A slender (550 long, 20 wide) larval nematode lacking lips was taken from the small intestine of an adult female eider, and identified as a *Capillaria* sp. Four specimens of a larval helminth with a buccal capsule and cervical papillae similar to those seen in male *T. somateriae* were examined. No body spines or spicules were seen, the specimens being identified as larval female *T. somateriae*. Measurements were as follows: length 2,000 (1,700–2,400); width (maximum) 96 (77–120); buccal capsule, length 19 (18–20), width 9 (6–11); distance of cervical papillae from anterior end 116 (111–120).

Berland (1961) described two larval *Anisakis* sp. [designated (1) and (11)] from Norwegian fish. A single specimen of an *Anisakis* sp. larva (1), as well as fish remains (muscles, vertebrae, eggs), was recovered from the gizzard of an adult female eider. The bird had been shot in summer when caplin (*Mallotus villosus* Muller) is extremely common in Newfoundland waters. It appears likely that the nematode had been released as fish that had been eaten by the bird was digested.

The free-living types which were probably ingested with the bird's food will not be dealt with in detail.

Acanthocephala

The small intestine of 101 (92%) of the birds contained *Polymorphus botulus* (89% of total number found in mid-half of intestine). Kulachkova (1958) noted this helminth in common eiders from the Kandalaksha Gulf and demonstrated a marked seasonal pattern of infection, Garden et al. (1964) finding differences in the intensity of infection in males and females. Data obtained during the present study tend to support Kulachkova's (1958) work with regard to the intensity of infections, with low numbers of Acanthocephala being found in the birds in fall and early winter (November, 46 per infected bird; December, 69), higher numbers in late winter and early spring (January, 104; March, 510), and low

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numbers again in the summer (June, 72; July, 37).

Breeding female birds tended to have low numbers of Acanthocephala (mean 25 per bird), this perhaps being related to the fact that at this time of year the birds either do not eat or subsist on plant materials (Kulachkova, 1958). A similar phenomenon was also noted in canvasback ducks [*Aythya valisineria* (Wilson)] in North America by Cornwell and Cowan (1963). During the present study, of the 23 breeding females examined, 10 had no food in their digestive tract, 7 contained fragments of *Mytilus edulis* L. shells, and 6 small quantities of items such as fish muscles, vertebrae, and eggs, shell fragments, small gastropods, and algae.

Considerable host reaction to the presence of this helminth was noted, small nodules forming wherever a proboscis was embedded. In several cases the small intestine of Acanthocephala-free birds was covered in such nodules indicating that the bird had at some time been infected with these helminths. Harrison (1955) used the term "nodular taeniasis" for this reaction. *P. botulus* has been cited as the cause of heavy mortality and epizootics in common eiders (*vide* McDonald, 1969). In four birds studied worms were found protruding through the wall of the small intestine. In two (male chick, juvenile male) there was little apparent damage, possibly due to the lesion being recent in origin, while in the other two (adult females) considerable damage was evident.

In the latter cases a perforation (approximately 1 cm diameter), with a thickened ring of tissue around it, was present. Shell fragments from ingested food gathered in these regions forming hard plugs of material, the viscera being held together by adhesions that must have reduced the total mobility of the gut. The birds in question were emaciated, the viscera appeared anemic, and the blood was less viscous than normal.

Haematozoa

Blood from two adult females was found to be infected with a *Plasmodium* sp. Specimens similar to this parasite have previously been found in waterfowl from the eastern United States and the Maritime provinces of Canada, but not in the common eider (Bennett, pers. comm.).

Ectoparasites

One unidentified species of mallophagan was found, on 24 (22%) of the birds examined. The chicks were louse-free.

The siphonapteran *Ceratophyllus garei* Rothschild, 1902, was recovered from three nests collected on St. Peter's Island, Labrador (area 6). Fox (1940) noted the occurrence of this flea in "eider down" from St. Mary's Island, Quebec.

The foregoing account gives some indication of the parasite burden of common eiders in Newfoundland. Species and numbers of parasites found in European common eider populations indicate that the numbers found in the present study are not abnormally high and that the data presented herein possibly represents the "normal" parasite burden of a wild population of common eider ducks.

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Eimeria megalostiedai sp. n. (Protozoa: Sporozoa) from the Wood Turtle, *Clemmys insculpta*, in Iowa[1]

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ABSTRACT: Oocysts of *Eimeria megalostiedai* sp. n. were isolated from a single wood turtle, *Clemmys insculpta*, in Iowa. The subspherical to broadly ovoid oocysts of *E. megalostiedai* averaged 13.9 (range 12.3 to 15.7) by 12.8 (range 10.3 to 14.7) microns. The broadly ellipsoidal sporocysts averaged 9.4 (range 7.8 to 10.1) by 5.1 (range 4.4 to 6.4) microns, and had an unusually large Stieda body (1.5 to 2.0 microns long) at one end. Oocyst and sporocyst residua were present. This appears to be the first report of an eimerian species from *Clemmys insculpta*.

During July 1972, the intestinal contents of a single wood turtle, collected in Iowa, were examined by us for coccidian oocysts. As a result, oocysts belonging to the genus *Eimeria* were found to be present, and are herein described as those of a new species. This appears to be the first report of an eimerian parasite from *Clemmys insculpta*, and therefore constitutes a new host record for the genus *Eimeria*. Wood turtles are extremely rare in Iowa, and the specimen which we examined is the first collected in over 30 years, the only other collection from the state being reported by Bailey (1941).

[1] Supported in part by Drake University Research Council grant No. 237-656.

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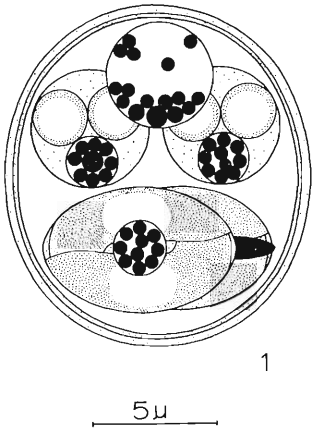


Figure 1. Sporulated oocyst of *Eimeria megalostiedai*.

Materials and Methods

The intestinal contents of the turtle were removed within 1 hr after the animal's death, placed in a specimen jar containing a shallow layer of 2.5% aqueous potassium dichromate, and stored at room temperature for 5 days to facilitate sporulation of the oocysts. The sample was then stored in a refrigerator until examined for the presence of oocysts. Oocysts were concentrated for examination by centrifugal flotation in Sheather's solution.

All observations were made with the aid of a compound microscope equipped with a bright-field condenser and achromatic objectives. All measurements were made with the aid of an ocular micrometer, and are herein expressed in microns; range measurements are in parentheses.

Results and Discussion

Eimeria megalostiedai sp. n.

Description

Oocysts (Fig. 1) subspherical to broadly ovoid. Outer surface of oocyst wall smooth; inner portion of wall appears slightly thinner and darker than outer portion. Wall of intact oocyst about 0.5 thick. Walls of sporulated oocysts wrinkle readily when placed in Sheather's solution. Micropyle and polar granule absent. Oocyst residuum present as spherical or ellipsoidal membrane-bound body containing several scattered spherical granules. Eighteen oocyst residual bodies ranged from 4.4 in minimum width or diameter to 8.8 in maximum length or diameter; the scattered spherical granules ranged from 0.5 to 1.5 in diameter. Thirty oocysts averaged 13.9 (12.3 to 15.7) by 12.8 (10.3 to 14.7); length/width ratio 1.1 (1.0 to 1.2). Sporocysts broadly ellipsoidal, with large Stieda body, 1.5 to 2.0 in length, present at one end. Substiedal body absent. Sporocyst residuum present as spherical membrane-bound body of about 2.5 to 3.0 in diameter, and containing densely packed spherical granules of about 0.5 to 1.0 in diameter. Position of sporocysts within oocyst variable, but may appear as illustrated. Thirty sporocysts averaged 9.4 (7.8 to 10.1) by 5.1 (4.4 to 6.4). One ellipsoidal refractile body of about 2.0 by 3.0 usually present in each sporozoite; nucleus not discernible.

TYPE HOST: Wood turtle, *Clemmys insculpta*.

TYPE LOCATION: Idewild Access on Cedar River, Northeast Quarter, Section 5, Floyd Township, Floyd County, Iowa.

Remarks

Thirteen species of *Eimeria* from turtles were reviewed by Pellerdy (1965). The present authors are aware of only four other species which have been reported from turtles in addition to those mentioned by Pellerdy. Three of these were described from turtles found in the United States; they are *Eimeria chelydrae* from the snapping turtle, *Chelydra serpentina* (Ernst et al., 1969), *E. scriptae* from the red-eared turtle, *Pseudemys scripta elegans* (Sampson and Ernst, 1969), and *E. paynei* from the gopher tortoise, *Gopherus polyphemus* (Ernst et al., 1971). The fourth species, *E. pseudemydis*, was described from *Pseudemys ornata* in British Honduras by Lainson (1968).

The sporulated oocysts of *Eimeria megalostiedai* may be distinguished morphologically from those of the other species of *Eimeria* reported from turtles by the following combination of characters: oocysts subspherical to broadly ovoid in shape, comparatively small, and with a length/width ratio of 1.1 (1.0 to 1.2); micropyle and polar granule absent, oocyst and sporocyst residua present; sporocysts broadly ellipsoidal with an unusually large Stieda body present at one end. Since no other eimerian parasite has been reported from the

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genus *Clemmys*, it is our opinion that *E. megalostiedai* may also be separated from the existing species reported from turtles on the basis of host specificity. The specific epithet given to this new parasite is of Greek derivation and means "large Stieda body."

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Description and Bionomics of *Octomyomermis troglodytis* sp. n. (Nematoda: Mermithidae) Parasitizing the Western Treehole Mosquito *Aedes sierrensis* (Ludlow) (Diptera: Culicidae)

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ABSTRACT: *Octomyomermis troglodytis* sp. n. (Nematoda: Mermithidae) is described from the larvae, pupae, and adults of the western treehole mosquito, *Aedes sierrensis* (Ludlow), in Marin County, California. The species possesses a barrel-shaped vagina, eight longitudinal chords, six cephalic papillae, two short separate spicules, and a cuticle without cross fibers. Specific characters include a slightly ventrally displaced mouth, an egg diameter approximately ½ the body width, and the pharyngeal tube approximately ¾ of the total body length. The adult tails never bear a terminal appendage. Parasitic development lasts from 20–22 days at 20 C and the adults remain in the moist rotted organic matter in the bottom of the treehole for most of the year. Infection occurs in March, April, and May and reached 38% in one treehole. This species could probably be mass-produced and used for biological control against *A. sierrensis* and possibly other treehole mosquitoes.

The western treehole mosquito, *Aedes sierrensis* (Ludlow), breeds in the winter and spring months in northern California. The adults feed on humans when natural hosts are not available and also serve as the intermediate host of *Setaria yehi* Dessert, a filarial parasite of deer in California. The nature of the larval habitat makes control of *A. sierrensis* difficult and a survey of its natural enemies revealed a mermithid nematode parasite (Sanders, 1972).

There are over 75 published reports of mosquitoes being attacked by mermithid nematodes; however, this is only the third account of a mermithid parasitizing mosquitoes in treeholes. Muspratt (1945) reported a mermithid attacking *Aedes* and *Culex* larvae in treeholes in Northern Rhodesia and Petersen and Willis (1969) cited a mermithid infecting *Orthopodomyia signifera* in a treehole in Louisiana. Neither of these nematodes was described or studied in detail.

The present account describes the mermithid from *A. sierrensis* and discusses its bionomics.

Materials and Methods

Treeholes in the coast live oak, *Quercus agrifolia* Nee, and California bay, *Umbellularia californica* (H. & A.), were sampled for infected

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larvae and pupae of *Aedes sierrensis* (Ludlow). Out of 18 treeholes sampled, only one in *Q. agrifolia* yielded mermithid parasitized stages of *A. sierrensis*. This treehole, which was 60 cm above the ground, held approximately 23 liters of water and had an east-southeast exposure. The treehole water consistently gave a pH reading slightly above 8.

Parasitized mosquitoes were maintained at 15–20 C in sterile treehole water with a pinch of Brewer's yeast until the nematodes emerged. The postparasitic juvenile nematodes were transferred to a small petri dish with a layer of sand in the bottom and held at 20–25 C. Mermithids were directly collected from the organic matter in the bottom of the treehole by carefully sorting through samples of debris removed by hand.

Adult mermithids were fixed in TAF (triethanolamine, formalin, and water) and processed to glycerin. Histological sections for determining the number of muscle fields and hypodermal chords were made with a freeze-microtome (cryostat) and sections were cut at 6 μ and stained with a 0.025% aqueous solution of methylene blue. Morphological studies on the parasitic juveniles were made on living material stained with a 0.05% aqueous solution of New blue R.

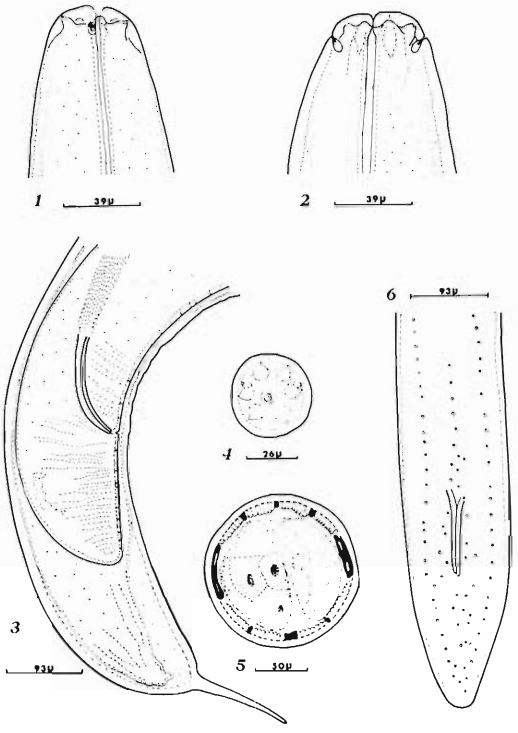
Results

The mermithid nematodes found parasitizing *Aedes sierrensis* were a new species and are described below in the genus *Octomyomermis* Johnson. In the quantitative portion of the description, the first figure is the mean of observations made and the range is given in parentheses. All measurements are in microns unless otherwise specified.

Octomyomermis troglodytis sp. n.
Mermithidae Braun, 1883.
Octomyomermis Johnson, 1963

ADULTS: Cross fibers lacking; eight hypodermal chords at midbody; six cephalic papillae in one crown; no labial papillae; mouth slightly displaced ventrally; amphids medium in size, without a commissure; tail bluntly rounded, without a mucron; pharyngeal tube extending ¾ of body length.

FEMALE (Figs. 2, 5, 9, 10) (n = 10): Length 12.6 (10.2–15.0) mm; greatest width 158 (120–165); head to nerve ring 236 (193–308); head to amphidial opening 10 (7–20); length amphidial pouch 4 (3–7); diameter amphidial opening 1.4 (0.6–2.6); distance posterior end to end of trophosome 99 (31–149); vagina barrel-shaped, length 83 (79–95); % vulva 57 (54–64); vulva protruding slightly, vulvar flap absent; egg diameter 81 (78–93); tail bluntly rounded.

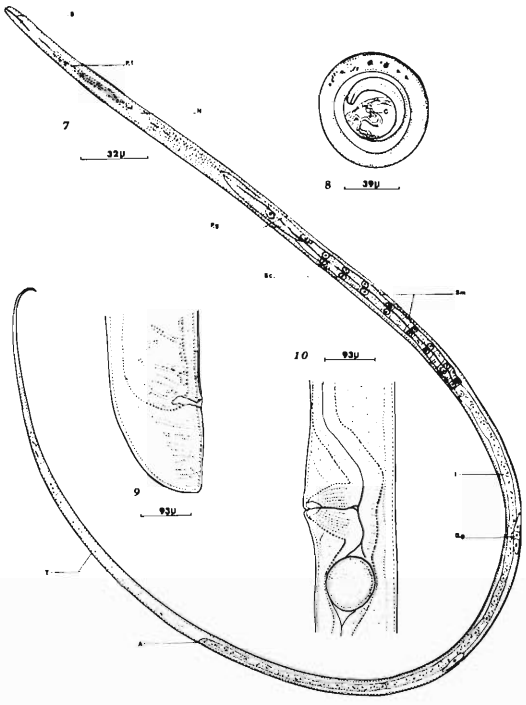


Figures 1–6. ***Octomyomermis troglodytis*** **sp. n. Lateral view of adult male head. 2. Ventral view of adult female head. 3. Lateral view of adult male tail with inner thin cuticle and outer thick cuticle with tail appendage of the postparasitic juvenile. 4. "En face" view of adult male. 5. Midbody cross section of adult female. 6. Ventral view of male tail with genital papillae.**

All females examined possessed a rudimentary anal opening and other evidence of maleness in the tail region such as weak copulatory muscles and abortive genital papillae. Such features are characteristic of intersexes in the Mermithidae.

MALE (Figs. 1, 3, 4, 6) (n = 8): Length 8.2 (7.2–9.1) mm; greatest width 117 (100–140); head to nerve ring 211 (193–254); head

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Figures 7–10. ***Octomyomermis troglodytis*** **sp. n. Lateral view of preparasitic juvenile. S, stylet; P.t., pharyngeal tube; N, nerve ring; P.g., penetration glands; Sm, stichosome; Sc, stichocytes; I, intestine; A, anus; T, tail. 8. Egg containing a preparasitic juvenile ready to hatch; C, molted cuticle. 9. Lateral view of adult female tail. 10. Lateral view of vulva and vagina of adult female.**

to amphidial opening 9 (7–13); length amphidial pouch 5 (4–7); diameter of amphidial opening 1 (1–3); length of tail 145 (130–162); width of anus 111 (100–126); spicules short, paired, parallel for most of their length, equal or subequal; length 104 (83–130); width 7 (6–9); tail blunt; genital papillae arranged in three broken rows, with the middle row becoming diffuse around the anus; each row contains from 19–23 preanal papillae and 10–15 postanal papillae.

POSTPARASITIC JUVENILE (Fig. 3): Leaves the host and molts twice before reaching the adult stage; length of tail appendage 106 (91–109).

PREPARASITIC JUVENILE (Figs. 7, 8): Length (620–650). This stage contains a well-developed stylet, a pharyngeal tube, nerve ring, stichosome with 16 stichocytes, a pair of penetration glands, intestine, gonad primordium, anus, and elongate tail. The anterior portion from the head to the base of the stichosome is roughly ⅓ of the total body length; the intestine makes up slightly more than ⅓ and the tail slightly less than ⅓ of the total length. The gonad primordium is located behind the junction of the pharynx and intestine. The alimentary tract is a single unit in this stage and separates only after the nematode begins its parasitic development. The thin-walled intestine lacks a lumen and is filled with granules. The anus is faint.

HOST: Body cavity of the larvae, pupae, and adults of *Aedes sierrensis* (Ludlow) (Diptera: Culicidae).

TYPES: Holotype (♀) and allotype (♂) deposited in the nematology collection at the University of California, Davis.

TYPE LOCATION: Treehole in *Quercus agrifolia* Nee near Novato, Marin County, California.

DIAGNOSIS: A small to medium-sized mermithid with a slightly ventrally displaced mouth, a barrel-shaped vagina, eight longitudinal chords, six cephalic papillae, two short separate spicules, and a cuticle without cross fibers.

The only other species in this genus is *O. itascensis*, which Johnson described from a chironomid. However, this nematode is considerably larger than *O. troglodytis*, possesses a terminal mouth, egg diameter approximately 1/7 of the body width, the pharyngeal tube extending approximately ¼ of the total body length, and both sexes with a rounded tail sometimes with a small terminal appendage. *O. troglodytis* possesses a slightly ventral mouth, egg diameter approximately ½ the body width, the pharyngeal tube extending approximately ¾ of the total body length, and the adult tails never with a terminal appendage. The hosts of these two species belong to different families of Diptera.

The diagnosis of *Octomyomermis* presented by Johnson (1963) is accepted here. The diagnosis given by Nickle (1972) is considered incomplete since he neglected to mention the number of cephalic papillae, the absence of cross fibers, or the number of hypodermal chords. Nickle used the nipplelike tip of the

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tails in both sexes as a generic character; however, Johnson stated that this character was only sometimes present.

Rubtsov (1968) recently described *Capitomermis crassiderma* which possesses the basic characters of the genus *Octomyomermis*. Members of this genus possess a thick cephalic layer of cuticle, posteriorly placed amphids, few genital papillae, and pointed tails in both sexes. All the above characters separate this species from *O. troglodytis*.

Bionomics

HOST: The western treehole mosquito deposits its eggs on the moist inner surface of rot holes above the water line in spring and summer. The fall rains wash the eggs into the filling treeholes and hatching occurs under favorable conditions. The second-, third-, and fourth-instar larvae may remain in the treehole for months, but pupation generally occurs in late winter and spring. The adults may be collected in all months except November and December and the female is long-lived and may take several blood meals before the deposition of each egg batch. This mosquito is usually a univoltine species; however, late spring rains may initiate a second generation.

PARASITE: *O. troglodytis* generally emerged from fourth-stage mosquito larvae, although pupae and adults were also infected. Parasitized larvae generally contained a single parasite but multiple infections also occurred. Upon emerging from the host (usually through the siphon), the nematodes entered the organic matter in the bottom of the treehole where they molted, mated, and oviposited. Under laboratory conditions, the final double molt occurred from 10–18 days after emergence with mating and oviposition occurring about 10–15 days after the final molt. The juveniles molted once within the egg and hatched about 15 days after oviposition.

Mermithids in various stages of their free-living development could be found throughout the year in the moist organic matter in the bottom of the treehole. By July, most of the eggs had been deposited and remained dormant over the fall and winter until the rains and warmer spring temperatures stimulated hatching.

Penetration of preparasitic juveniles into mosquito larvae was observed in the laboratory. The extremely active preparasitic juveniles, which spend most of their time at the surface of the water, made contact with resting *A. sierrensis* larvae. One parasite penetrated in the saddle area. Remaining motionless except for movement of its stylet, the mermithid pressed its head against the host's cuticle and pharyngeal gland secretions probably were expelled through the stylet onto the insect's cuticle. In about 10 min, a small hole was made in the mosquito's cuticle and the nematode suddenly straightened out and entered the hemocoel within a few seconds. Examination of newly penetrated juveniles showed that their penetration glands had collapsed, indicating their probable use for entry into the host.

The nematodes initiated growth in the host at the base of the siphon and then moved anteriorly after 8–10 days. The entire developmental period lasted from 20–22 days at 20 C. All hosts died soon after the nematodes emerged and detailed examinations indicated fat body depletion.

The number of parasitized larvae collected during the winter and spring of 1972 is shown in Figure 11. Most infected larvae were found in late March, April, and early May, when the mosquito populations were beginning to decline. In May, the incidence of parasitism reached 38%.

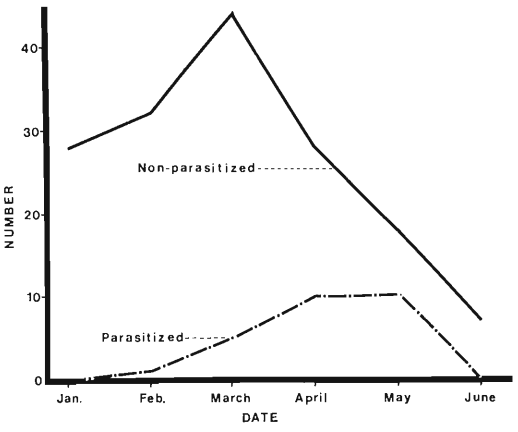


Figure 11. The number of mosquito larvae collected and parasitized by *Octomyomermis troglodytis* sp. n. during the winter and spring of 1972.

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Discussion

Other pathogens of *A. sierrensis* in treeholes included the fungus, *Beauveria tenella*, and the ciliated protozoan, *Tetrahymena* sp. The latter organism was occasionally found developing together with *O. troglodytis* (Sanders, 1972).

A second mermithid species was recovered from sciarid larvae living in the bottom of the treehole during the summer months. This parasite was distinct from *O. troglodytis* and was never recovered from *A. sierrensis*.

Since *A. sierrensis* is often a problem in urban areas near gardens and parks, *O. troglodytis* may be considered a possible means of control for this and perhaps other treehole culicids. Although the natural distribution of this mermithid is apparently limited, techniques have been developed for the artificial propagation of mermithids of mosquitoes (Muspratt, 1965; Petersen and Willis, 1972) and it may be possible to mass produce *O. troglodytis* for release in uninfested treeholes. The mermithid, *Reesimermis nielseni* Tsai and Grundman, that is now being used for the biological control of mosquitoes, will not tolerate the water in treeholes and cannot be used against these species (Chapman, pers. comm.).

In the descriptive portion of this study, emphasis was placed on the morphology of the preparasitic (= infective) juveniles. In most mermithid descriptions, little, if any, mention is made of this stage. However, extensive examinations of the preparasitic juveniles of different mermithid species representing several genera showed that this stage contains characteristic taxonomic features. The length and shape of the stylet, shape of the penetration glands, length of the stichosome and intestine, position of the gonad primordium, presence of the anus, and length of the tail are basic diagnostic characters that can definitely be used in mermithid taxonomy.

Acknowledgments

We would like to thank Drs. H. C. Chapman and J. J. Petersen of the Entomology Research Service, Lake Charles, Louisiana, for sending us other mermithids from mosquitoes for comparative studies. Grateful appreciation is also extended to G. M. Thomas for help in various aspects of this study and to Steven Hartman for assistance with the drawings.

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Calentinella etnieri gen. et sp. n. (Cestoidea: Caryophyllaeidae) from *Erimyzon oblongus* (Mitchill) (Cypriniformes: Catostomidae) in North America

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ABSTRACT: The new genus and species is described from the creek chubsucker, *Erimyzon oblongus* (Mitchill), in western Tennessee. *Calentinella* differs from other single-gonopored genera by having an ovary without posterior arms, lacking an external seminal vesicle, and having a weakly developed cuneiform scolex. A key to the single-gonopored genera in the family Caryophyllaeidae is presented.

While working on the cytology or caryophyllid cestodes in the laboratory of Dr. A. Jones, University of Tennessee, from August 1967 to August 1968 a large number of new species of these cestodes were discovered in the catostomid fishes of Tennessee and neighboring states. Two of these new species have already been described (Mackiewicz, 1972); this paper concerns a new genus collected during that year and additional material contributed by Dr. D. Etnier, University of Tennessee.

Although the entire sample consists of only five gravid and three immature worms the species is so distinct that its description is warranted.

Specimens were fixed in either Carnoy's fixative or 10% formalin and stained in Fuelgen's stain or Semichon's carmine as whole mounts. Sections were prepared from a portion of a carmine-stained whole mount, with eosin counterstain. Measurements are in microns unless otherwise stated; drawings were made with the aid of a microprojector.

Order Caryophyllidea Van Beneden (in Carus, 1863)

Family Caryophyllaeidae Leuckart (in Lühe, 1919)

Calentinella gen. n.

GENERIC DIAGNOSIS: Caryophyllaeidae. Scolex cuneiform, aloculate. Cirrus joining uterovaginal canal. Ovary lacking posterior arms, U-shaped. Uterus not extending anteriorly beyond cirrus sac. Preovarian vitellaria median and lateral. Postovarian vitellaria present. External seminal vesicle absent. Type species: *Calentinella etnieri* sp. n.

Remarks

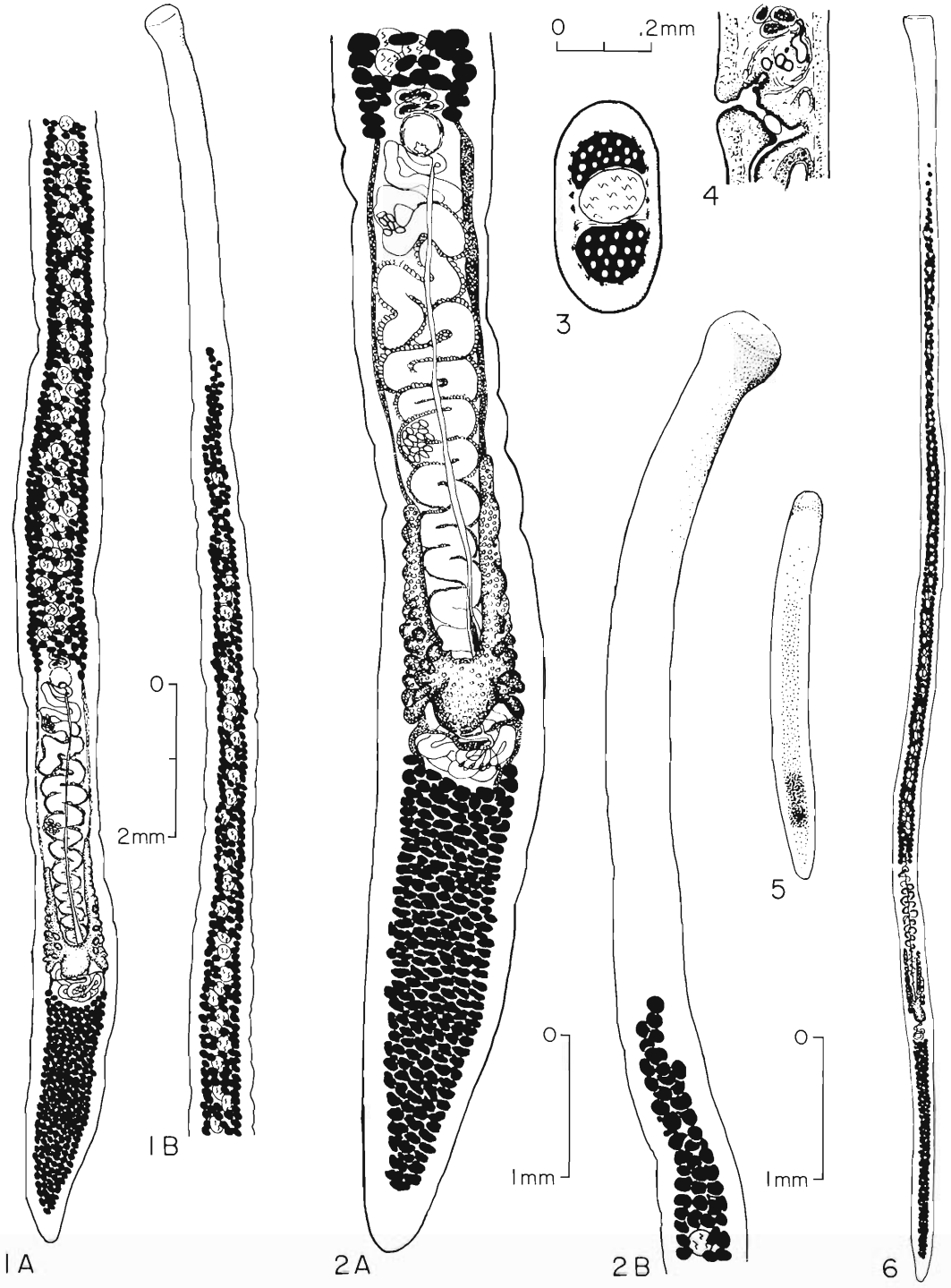
Calentinella can easily be separated from the other genera in the family having a single gonopore, namely *Archigetes* Leuckart, 1878, *Biacetabulum* Hunter, 1927, *Pliovitellaria* Fischthal, 1951, *Bialovarium* Fischthal, 1953, *Isoglaridacris* Mackiewicz, 1965, and *Penarchigetes* Mackiewicz, 1969. It differs from all of these genera, except *Bialovarium*, by having an ovary without posterior arms; except *Penarchigetes*, by lacking an external seminal vesicle; and except *Isoglaridacris*, whose scolex is basically of the same type, i.e., cuneiform but is expanded and usually loculate. It also differs from these three genera by having median vitellaria and additionally from *Bialovarium* by having postovarian vitellaria. The long postovarian vitelline region is reminiscent of *Pliovitellaria* but the scolex differences as well as those of ovarian morphology and presence of an external seminal vesicle serve to separate this genus from *Calentinella*.

It is not possible to make an adequate comparison between the new genus and *Paracaryophyllaeus* Kulakovskaya, 1961, because descrip-

→

Figures 1–6. 1A, B. Whole worm, holotype. 2A. Posterior portion. 2B. Scolex. 3. Cross section through middle of worm 16 mm long. 4. Midsagittal section through cirrus illustrating the hermaphroditic duct that connects the gonopore with the ejaculatory duct and uterovaginal canal. 5. Immature worm. 6. Whole worm.

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tions of the latter do not note the placement of the inner longitudinal muscles, whether an external seminal vesicle is present or absent, or the number of gonopores. I am unable to observe any of these important generic features on the single specimen of *Paracaryophyllaeus* in my possession. However, on the basis of an H-shaped ovary and anterior position of the uterus (i.e., anterior of the cirrus) of *Paracaryophyllaeus* as well as a great difference in the distribution of the two genera—palearctic Cobitidae for *Paracarypohyllaeus* and nearctic Catostomidae for *Calentinella*—the two genera are regarded as distinct.

This genus is named in honor of Dr. R. Calentine, of the University of Wisconsin, for his many significant contributions to our understanding of caryophyllid biology and systematics; the generic name is feminine in gender. The following key will serve to separate the single-gonopored Caryophyllaeidae from each other.

Key to the single-gonopored Caryophyllaeidae genera from vertebrate hosts

1. Preovarian vitellaria in two lateral rows; postovarian vitellaria present or absent .. 2
2. Ovary in shape of letter U; postovarian vitellaria absent; external seminal vesicle present *Bialovarium* Fischthal, 1953
2. Ovary in shape of letter H, dumbbell, or with posterior arms joined; postovarian vitellaria present or absent; external seminal vesicle present or absent 3
3. Scolex cuneiform–loculate; ovary with posterior arms joined or nearly so in same species; postovarian vitellaria present or absent; external seminal vesicle present *Isoglaridacris* Mackiewicz, 1965
3. Scolex bothrioloculodiscate [i.e., with two median bothria, four lateral loculi, and a sincipital plate or terminal disc; corresponding to the "II" type of Hunter (1930)]; postovarian vitellaria present .. 4
4. External seminal vesicle present, large; scolex not expanded laterally, with a large prominent terminal disc that is clearly wider than the deep bothria; in Cyprinidae *Archigetes* Leuckart, 1878
4. External seminal vesicle absent; scolex expanded laterally, with a small poorly developed terminal disc that is generally as wide as the shallow bothria; in Catostomidae .. *Penarchigetes* Mackiewicz, 1969
1. Preovarian vitellaria median as well as lateral; postovarian vitellaria present 5
5. Uterus extending anterior of cirrus by more than one cirrus sac width; scolex bothriate, acetabulate, or discate *Biacetabulum* Hunter, 1927
5. Uterus extending to cirrus or no more than one cirrus sac width anterior of it; scolex various but not acetabulate 6
6. Scolex bothrioloculodiscate; ovary dumbbell-shaped; small worms generally less than 2 or 3 mm long; in Cyprinidae *Archigetes* Leuckart, 1878
6. Scolex cuneiform or clavate; ovary conspicuously H-shaped or lacking posterior arms; in Cyprinidae and Catostomidae .. 7
7. External seminal vesicle present; ovary distinctly H-shaped; scolex clavate or clavoloculate; in Cyprinidae *Pliovitellaria* Fischthal, 1951
7. External seminal vesicle absent; ovary lacking posterior arms; scolex cuneiform; in Catostomidae *Calentinella* gen. n.

Calentinella etnieri gen. et sp. n. Etnier's caryophyllid (Figs. 1–6)

SPECIFIC DIAGNOSIS (unless otherwise specified means are based on whole mounts of five gravid individuals from three fish; ranges in parentheses; one work sectioned): Worms 20.3 (12.7–29.6) mm long by 630 (330–1,000) wide at gonopore. Body filiform. Scolex small, cuneiform, aloculate, generally as wide as, or less than, greatest body width. Neck long. Inner longitudinal muscles form line of large fascicles. Outer longitudinal muscles apparently absent. Testes generally round to oval, begin 3.8 (2.8–6.3) mm from scolex apex, extend to cirrus sac, number 74 (59–86), measure 155 (75–225) in diameter ($N = 24$: three most anterior and posterior from each of four worms). External seminal vesicle absent. Internal seminal vesicle present. Cirrus sac 200 (100–320) in diameter, muscles weakly developed, contained in body width at gonopore 2½ to 3½ times, generally more than an ovary length from ovary. Gonopore large, conspicuous. Preovarian vitelline follicles round to irregular in shape, smaller than testes, chiefly in

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lateral but also in median fields, begin anterior to testes, 2.8 (2–5) mm from scolex apex, generally extend to cirrus sac, occasionally to ovary. Previtelline distance contained in length of worm 7.4 (6–9.2) times and represents 13.8 (10.9–16.7) per cent of worm length. Postovarian vitelline field from 1½ to 3 times ovary length. Postgonopore distance 6.3 (4.8–8.3) mm, contained in length of worm 3.3 (2.3–4) times and represents 32.2 (24.9–43) per cent of worm length. Ovary with long anterior arms but lacking posterior arms, commissure large and swollen; follicular to lobulate, 1.3 (1–1.8) mm long (from anterior tip to most posterior lobe or follicle). Seminal receptacle absent. From six to eight osmoregulatory canals at midbody.

Immature worms ($N = 3$) ranged in size from 2.5 (Fig. 5) to 11.5 mm.

SPECIMENS STUDIED: 5 gravid, 3 immature.

HOSTS: Creek chubsucker, *Erimyzon oblongus* (Mitchill), type host (Cypriniformes: Catostomidae).

LOCATION: Intestine; attached, but weakly so.

LOCALITIES: Tennessee: Haywood Co., Mud Creek (Hatchie River drainage), 1 mile west of Union Tenn. route 54 (type locality) 16 April 1969, collected by Dr. David Etnier; Obion Co., Indian Creek near town of Samburg on east side of Reelfoot Lake, 12 March, 17, 18 July 1968, Mississippi River drainage.

TYPE SPECIMENS: Holotype, USNM Helm. Coll. No. 72621. Paratype (1) USNM Helm. Coll. No. 72622.

Supplementary material (three slides) consisting of a single whole mount and sections include USNM Helm. Coll. Nos. 72623–4.

Remarks

Eggs were not measured because all were collapsed.

This species has some unusual features. Obvious is the extensive postovarian vitellaria (POV) similar to the condition found in only two other genera—*Pliovitellaria* and *Wenyonia* Woodland, 1923. As a result of this extensive POV development the gonopore is more anterior than in other genera (Fig. 6); this anterior placement is further accentuated because of the exceptionally long uterus (Figs. 1A, 2A). While the ovary appears basically like that of *Spartoides* Hunter, 1929, the most posterior follicles do not extend beyond the ovarian commissure as is so often the case in that genus. The internal seminal vesicle is not as well developed as that found in *Caryophyllaeus*, for example (Fig. 4). Preovarian vitellaria of small worms (Figs. 3, 6) may appear to be chiefly in two rows because of the large size of the testes and narrow body; however, median vitellaria are clearly present in the posterior portion of the preovarian vitelline field of small worms and throughout the field in larger worms (Figs. 1A, B). Furthermore, the most anterior vitellaria of all specimens do not extend in two lateral rows ahead of the testes, the condition usually characteristic of species having lateral vitellaria [e.g., *Glaridacris laruei* (Lamont) or *Isoglaridacris folius* Frederickson and Ulmer]. Perhaps most unusual is the enormous variation in size of mature worms as shown by Figures 2A, B, 5, and 6 which were drawn to the same scale. These worms came from different fish, however. This striking difference emphasizes the care that must be used in using size as a taxonomic character in the Caryophyllidea.

The intensity of infection varied from one to four worms per fish; concurrent infections included *Biacetabulum biloculoides* Mackiewicz and McCrae, 1965. Of 20 fish from Indian Creek averaging in size 146 mm (100–185) four were infected.

The species is named for Dr. D. Etnier, ichthyologist from the University of Tennessee, who so generously assisted in the collection of this species and others now under study.

Acknowledgments

The author gratefully acknowledges the assistance of Dr. D. Etnier and Mr. B. Smythe for assistance in collecting hosts and Mr. Lewis Burrus of the Tennessee Department of Fish and Game for facilities at Reelfoot Lake.

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Two New Species of *Pharyngodon* Diesing, 1861 (Nematoda: Oxyuridae) from Lizards in West Texas

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ABSTRACT: *Pharyngodon kirbii* sp. n. and *P. mudgi* sp. n. are described from *Cnemidophorus scalaris* and *Coleonyx brevis* from West Texas. The former species is unique in that the males possess two pairs of sessile papillae on the genital cone; the latter species can be distinguished from other species in which females possess a spinous tail by the anterior location of the vulva in the female and the reduced length of the tail in the male. The occurrences of *P. cnemidophori* in *Cnemidophorus tigris* and *P. warneri* in *Cnemidophorus inornatus* represent new host and distribution records. *P. paratectipenis* is transferred to the genus *Spauligodon*. *Skrjabinodon medinae* is proposed as a new combination for *P. medinae*. A key is provided for species of the genus *Pharyngodon*.

During a survey of the helminths of reptiles from West Texas several species of lizards were found to be infected with two undescribed and two previously described species of the genus *Pharyngodon* Diesing, 1861.

The genus *Pharyngodon*, as presently understood, contains 20 species, three of which are known only from females, *P. batrachiensis* Walton, 1929, *P. boulengerula* Ubelaker, 1965, and *P. polypedatis* Yamaguti, 1941. Since *Pharyngodon* has been divided into several genera based on characters of the males—*Spauligodon* Skrjabin, Schikhobalova, and Lagodovskaja, 1960; Skrjabinodon Inglis, 1968; and the closely related genus *Parathelandros* Baylis, 1930, as redefined by Inglis, 1968—it is only for convenience that the species *P. batrachiensis*, *P. boulengerula*, and *P. polypedatis* are retained in the genus *Pharyngodon*.

The worms were fixed in alcohol and cleared in lactophenol or glycerine for study. All measurements are in microns unless otherwise noted. The range is followed by the average in parentheses.

Pharyngodon kirbii sp. n. (Figs. 1–6)

Description

Nematodes white with straight, stout bodies. Cuticle with distinct transverse striations extending from behind lips to level of anus. Mouth surrounded by three lips: one dorsal with two papillae, two subventral each with two papillae, and an amphid. Lateral alae present in males only. Measurements from four male and 11 female specimens.

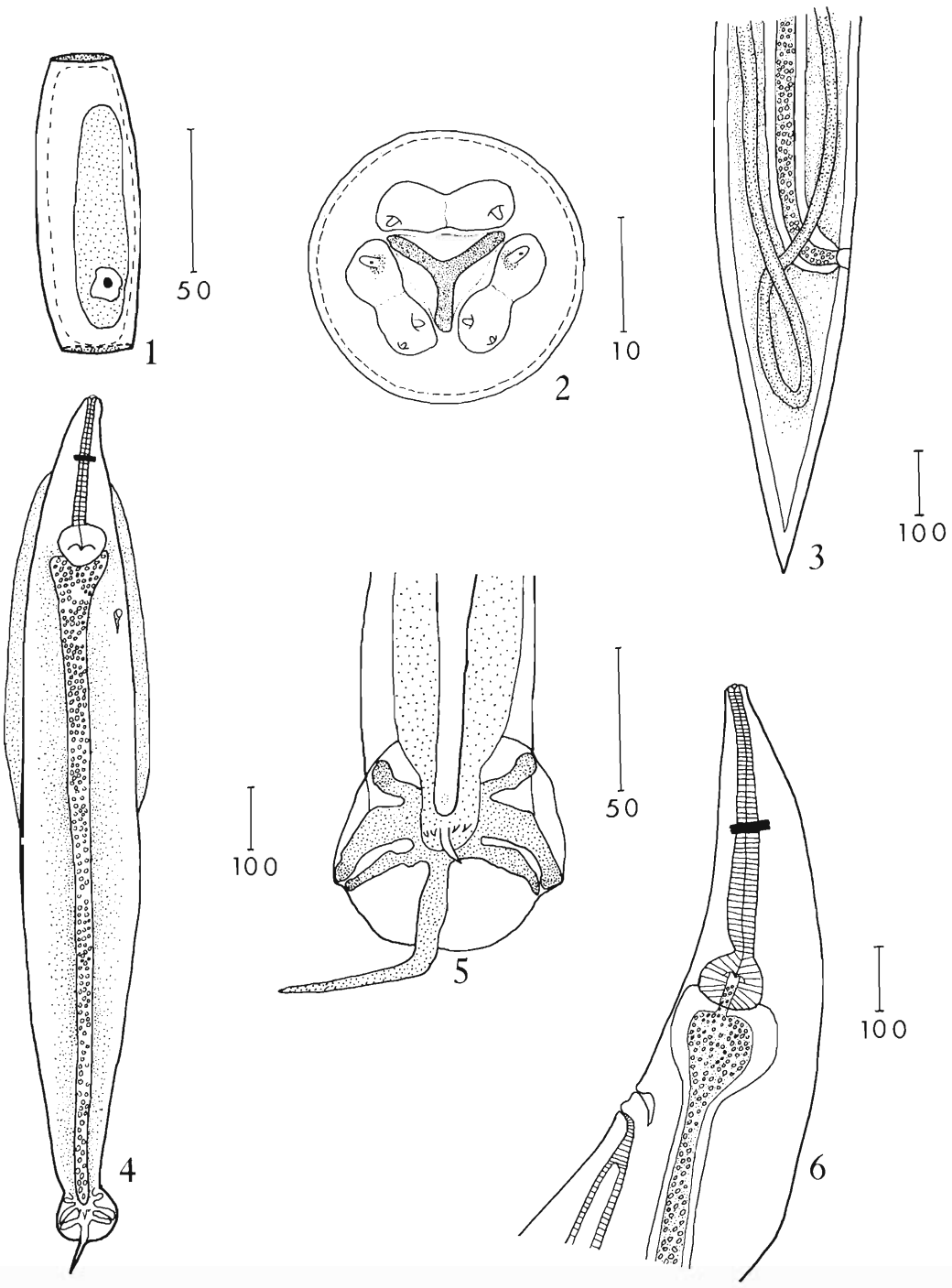
MALE: Mature specimens 1.17 to 1.59 mm (1.42 mm) long, 98 to 158 (129) wide. Esophagus 299 to 321 (307) long, posterior bulb 50 to 59 (54) wide. Nerve ring 73 to 75 (74) and excretory pore 438 to 475 (460) from anterior end, respectively. Lateral alae extend from average of 150 from anterior end to just past middle of body. Bursa 65 to 75 (69) long, tail to anus 107 to 111 (109), tail free of bursa 48 to 62 (57). Five pairs of papillae present, three pedunculate, two sessile. Well-developed genital cone located between two pairs of sessile papillae. The three pairs of pedunculate papillae are located in the usual manner: one preanal, two postanal. Spicule and gubernaculum are absent or poorly chitinized.

FEMALE: Gravid females 2.84 to 4.35 mm (3.39 mm) long, 226 to 409 (343) wide. Esophagus 431 to 584 (523) long, posterior bulb 66 to 82 (74) wide. Nerve ring 96 to 117 (108) and excretory pore 540 to 817 (646) from anterior end, respectively. Vulva, with protruding lips located just posterior to excretory pore, 591 to 869 (710) from anterior end.

→

Figures 1–6. ***Pharyngodon kirbii.*** **(Scales in microns.) 1. Egg. 2. *En face* view of female. 3. Posterior end of female. 4. Adult male. 5. Posterior end of male showing arrangement of caudal papillae. 6. Anterior end of female.**

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Muscular vagina directed posteriorly. Uterus didelphic, one uterine branch directed posteriorly, the other anteriorly. Uteri, oviducts, and ovaries coil about intestine to about level to excretory pore. Ova cylindrical, bioperculate, 108 to 116 (112) long, 33 to 38 (35) wide. Ova in multicellular stage when oviposited. Tail to anus 481 to 657 (542). Tail tapers gradually to a point.

TYPE HOST: *Cnemidophorus scalaris* (Cope, 1892).

TYPE LOCALITY: Stairway Mountain, Black Gap Wildlife Management Area, Brewster County, Texas.

SITE OF INFECTION: Posterior region of the large intestine.

HOST: Dallas Museum of Natural History Herp. Coll. No. 916. Holotype male and allotype female: USNM Helm. Coll. No. 72534. Paratypes: USNM Helm. Coll. No. 72618.

Remarks

The genus *Pharyngodon*, as it stands at present, can be divided into a larger group characterized by females with subulate tails and a smaller group by females with short, gradually tapering tails. *Pharyngodon kirbii* is a member of the latter group as are *P. warneri* Harwood, 1932, *P. travassoi* Pereira, 1935, *P. cesarpintoi* Pereira, 1935, *P. papilliocauda* Hannum, 1943, and *P. cnemidophori* Read and Armein, 1954.

Pharyngodon kirbii is unique in having two pairs of sessile papillae located on either side of the genital cone. The preanal pedunculate papillae in *P. kirbii* are elongate, while those in *P. warneri* and *P. travassoi* are quite reduced. *Pharyngodon kirbii* has prominent lateral alae which are not reported from *P. papilliocauda* and *P. warneri*. The lack of numerous papillae on the female tail distinguishes it from *P. papilliocauda*.

Pharyngodon kirbii most closely resembles *P. cnemidophori* and is distinguished from it primarily by much smaller females and smaller eggs, as well as the above-mentioned sessile caudal papillae.

Pharyngodon mudgi sp. n.
(Figs. 7–11)

Description

Nematodes white with straight, stout bodies. Cuticle with distinct transverse striations extending length of body. Mouth surrounded by three lips: one dorsal, two subventral. Lateral alae present in males only. Measurements from three males and one female.

MALE: Mature specimens 1.16 to 1.49 mm (1.33 mm) long, 175 to 183 (178) wide. Esophagus 234 to 270 (256) long, posterior bulb 59 to 71 (64) wide. Nerve ring 60 to 65 (63) and excretory pore 321 to 365 (343) from anterior end, respectively. Lateral alae from just posterior to lips to about middle of body. Bursa 59 to 72 (64) long, contains three pairs of pedunculate papillae: one preanal, two postanal. The preanal pair is distinctly forked. Well-developed genital cone present. Tail to anus 71 to 98 (84), tail free of bursa 44 to 57 (52). Spicule and gubernaculum are absent or poorly chitinized.

FEMALE: Mature female 4.01 mm long, 329 wide. Esophagus 423 long, posterior bulb 65 wide. Nerve ring 241 and excretory pore 256 from anterior end, respectively. Vulva with protruding lips located anterior to esophageal bulb and just posterior to excretory pore, 292 from anterior end. Muscular vagina directed posteriorly. Uterus didelphic, one uterine branch directed anteriorly, and the other posteriorly. Uteri, oviducts, and ovaries extend to about level of excretory pore. Ova ellipsoid and slightly flattened on one side, 103 to 106 (105) long and 32 to 42 (35) wide. Ova are operculate and in multicellular stage when oviposited. Tail to anus 423, tail covered with numerous spines.

TYPE HOST: *Coleonyx brevis* Stejneger, 1893.

TYPE LOCALITY: Black Gap Wildlife Management Area, 1 mile S headquarters on Farm Rd. 2627, Brewster County, Texas.

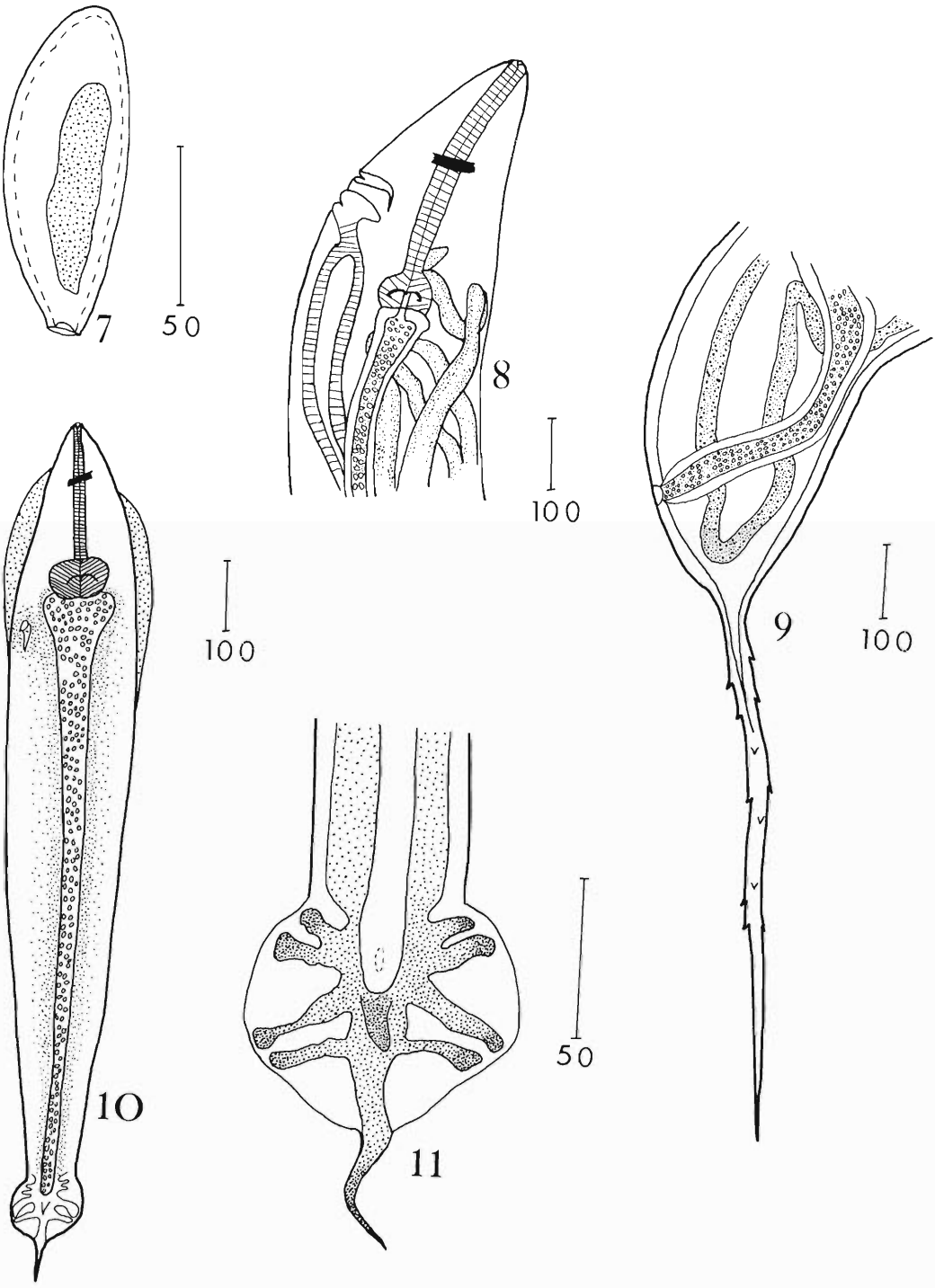
SITE OF INFECTION: Posterior region of the large intestine.

HOST: Dallas Museum of Natural History

→

Figures 7–11. ***Pharyngodon mudgi.*** **(Scales in microns.) 7. Egg. 8. Anterior end of female. 9. Posterior end of female. 10. Adult male. 11. Posterior end of male showing arrangement of caudal papillae.**

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Herp. Coll. No. 348. Holotype male and allotype female: USNM Helm. Coll. No. 72535.

Remarks

Four species of the genus *Pharyngodon* are known to possess females with subulate tails bearing spines: *P. spinicauda* Dujardin, 1845, *P. kartana* Johnston and Mawson, 1941, *P. yucatanensis* Chitwood, 1938, and *P. boulengerula* Ubelaker, 1965. Two species have been described from members of the Geckonidae, *P. yucatanensis* from *Coleonyx elegans* Gray in Mexico and *P. geckinis* Liu and Wu, 1941 from *Gecko gecko* (Linnaeus, 1758) in China.

Pharyngodon mudgi can be distinguished from all species listed above by two readily observable characters. The excretory pore and vulva in *P. mudgi* are located anterior to the esophogeal bulb in contrast to the other species (with possible exception of *P. geckinis*) where these structures are located at the level of or posterior to the esophageal bulb. The presence of spines on the tail of *P. mudgi* readily distinguishes this species from *P. geckinis*.

Pharyngodon spinicauda, *P. yucatanesis*, and *P. kartana* are all characterized by males in which the tails are at least three times the length of the bursa. The male tail in *P. mudgi* is shorter, less than two times the length of the bursa. *Pharyngodon mudgi* and *P. kartana* are similar in that both species possess forked preanal papillae.

The distribution and ecology of the three species of *Cnemidophorus* in the collection area has been discussed by Milstead (1957). The type host of *Pharyngodon kirbii*, *C. scalaris* is restricted to the rugged upland plateaus in sotol–lechugiulla plant associations. According to Milstead, *C. scalaris* is restricted in distribution by the more aggressive *C. tigris* which ranges over the creosote bush flats, particularly in draws with gentle slopes. Specimens of *P. cnemidophori* were obtained from *C. tigris* only at a single locality in Javelina Creek bed. The hillsides are occupied by a third species, *C. inornatus*, from which specimens of *P. warneri* were recovered. The occurrences of *P. cnemidophori* in *C. tigris* and *P. warneri* in *C. inornatus* represent new host and distribution records.

Discussion

The genus *Pharyngodon* Diesing, 1861, was restricted by Skrjabin et al. (1960) to species in which the caudal alae of males enclose all the caudal papillae. Based on the arrangement of the caudal alae and caudal papillae, these authors removed several species to *Spauligodon* Skrjabin, Schikhobalova, and Lagodovskaja, 1960, and *Parathelandros* Baylis, 1930.

Pharyngodon paratectipenis Chabaud and Golvan, 1957 (*P. tectipenis* sensu Calvente, 1948 nec. *P. tectipenis* Gedoelst, 1919) does not appear to belong to the genus *Pharyngodon* since the males possess a pair of posterior caudal papillae that lie outside the caudal bursa. We are, therefore, proposing a new combination, *Spauligodon paratectipenis*. *Pharyngodon medinae* Calvente, 1948, lacks caudal alae and possesses a single pair of sessile preanal papillae. We are thus proposing a second new combination, *Skrjabinodon medinae*. *Pharyngodon neyrae* Calvente, 1948, appears to be distinctively different from other members of *Pharyngodon*. Although all caudal papillae are contained in the caudal alae as in the genus *Pharyngodon*, the nature of the lateral alae, arrangement of the papillae, and shape of the egg are not consistent with other members of the genus. If this species can be restudied to confirm these characters, it should be removed to a new genus.

The key presented below represents the genus as now delineated and is modified from a key presented by Chabaud and Golvan (1957). Species known only from female specimens are not included in the key.

1A. Tail of mature female subulate 2
1B. Tail of mature female tapers gradually to a point 11
2A. Tail of female with spines 3
2B. Tail of female without spines 6
3A. Tail of male at least three times as long as length of bursa 4
3B. Tail of male less than two times as long as bursa, vulva opens anterior to the esophageal bulb *P. mudgi* sp. n.
4A. Preanal papillae forked in male *P. kartana* Johnston and Mawson, 1941
4B. Preanal papillae not forked in male .. 5
5A. Male with spicule; eggs average 155 μ long by 51 μ wide *P. spinicauda* Dujardin, 1845

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5B. Male without spicule; eggs average 116 μ long by 34 μ wide *P. yucatanensis* Chitwood, 1938
6A. Tail of male at least twice as long as length of bursa 7
6B. Tail of male about as long as bursa *P. hindlei* Thapar, 1925
6C. Tail of male definitely shorter than bursa 10
7A. Two pairs of preanal papillae *P. geckinis* Liu and Wu, 1941
7B. One pair of preanal papillae 8
8A. Narrow lateral alae in male *P. tiliquae* Baylis, 1930*
8B. Relatively wide lateral alae in male .. 9
9A. Male with three pairs of caudal papillae *P. inermicauda* Baylis, 1923
9B. Male with four pairs of caudal papillae *P. neyrae* Calvente, 1948
10A. Two pairs of preanal papillae, most anterior pair of postanal papillae forked *P. australis* Johnston and Mawson, 1944*
10B. One pair of preanal papillae, no forked papillae present *P. mamillatus* (Linstow, 1897)
11A. Many irregular papillae on tail of female *P. papilliocauda* Hannum, 1942
11B. No papillae on tail of female 12
12A. Tail of male obviously longer than length of bursa *P. cesarpintoi* Pereira, 1935
12B. Tail of male less than or equal length of bursa 13
13A. Preanal papillae greatly reduced in length 14
13B. All caudal papillae about equal in length 15
14A. Lateral alae present in males, spicule obvious *P. travassoi* Pereira, 1935
14B. Lateral alae absent on males, spicule absent or poorly chitinized *P. warneri* Harwood, 1932
15A. Two pairs sessile papillae located around genital cone *P. kirbii* sp. n.
15B. No sessile papillae found in region of genital cone *P. cnemidophori* Read and Armein, 1953

* Mawson (1958) reports many similar characters in *P. tiliquae* and *P. australis*. Their status as distinct species is uncertain at the present time.

Acknowledgments

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Infection Dynamics of the Cattle Parasite, *Ostertagia ostertagi*, in Sheep

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ABSTRACT: No patent infections were produced in 17 lambs inoculated orally with infective larvae of the bovine parasite, *Ostertagia ostertagi*. Larvae did exsheath, enter gastric pits, undergo development to the adolescent stage, and produce abomasal lesions. Adults and a mixture of L3, L4, and adolescent fifth stages recovered from calves and transferred orally to lambs produced patent infections. A mixture of L3 to adolescent stages recovered from lambs and transferred orally to lambs and calves did not produce patent infections.

The literature dealing with the incidence of gastrointestinal nematode parasites of sheep is replete with references to the recovery of *Ostertagia ostertagi*, a nematode parasite of the abomasum in cattle. Paradoxically, experimental inoculation of sheep with the infective larvae of *O. ostertagi* has failed to produce patent infections (Herlich, 1958; Bessanov, 1959; Pandey, 1971). Only five or fewer lambs were used in each of those studies; therefore, it seemed important from an epizootiologic standpoint to investigate more fully the infective potential of *O. ostertagi* in sheep.

Materials and Methods

Helminth-free Dorset lambs from 6 to 8 months old and Holstein-Friesian 4-month-old calves were used. The parasite was the OOH isolate of *O. ostertagi* that has been maintained at this institute for 8 years.

EXPERIMENT 1—ADMINISTRATION OF INFECTIVE LARVAE TO LAMBS: Ten lambs were each inoculated orally with a single dose of 50,000 infective larvae, and two others with 500,000 larvae. Feces were collected weekly for 3 months and examined for nematode eggs by direct centrifugal flotation in zinc sulfate. Five additional lambs were inoculated with a single dose of 37,200 to 200,000 infective larvae each (Table 1). All abomasa were excised, washed thoroughly, and exposed to pepsin–hydrochloric acid digestion for 8 hr. Worms recovered from duplicate 5% aliquots were used to determined the stages of development and numbers recovered. Stages recovered were identified by comparison with the descriptions of Douvres (1956) and Rose (1969). Pieces of abomasum were excised and fixed for histological study.

EXPERIMENT 2—TRANSFER OF ADULT WORMS FROM CALVES TO LAMBS: Two calves were each inoculated with 100,000 infective larvae and killed 35 days later. Abomasal contents of each were collected individually in warm saline, and an aliquot was removed for determination of the stages and numbers of worms present. The material was allowed to settle while kept in an incubator for 30 min, and the supernatant fluid was decanted. Half the sediment, containing worms, was administered by drenching gun to each of four lambs; two were each given 13,000 adults and the two others were each given 37,000. Fecal samples were collected after 24 hr and weekly thereafter. Lambs were killed individually 12, 27, 45, and 84 days after the oral transfer.

EXPERIMENT 3—TRANSFER OF PARASITIC LARVAE FROM CALF TO LAMB: Two calves were each inoculated with 50,000 infective larvae and killed 12 days later to recover worms from the contents. These worms, comprising a mixture of L4 and adolescents, were administered orally to two lambs—2,500 and 3,500, respectively. Fecal samples were collected daily for 22 and 30 days after the transfers, respectively, and the lambs were then killed for recovery of worms.

EXPERIMENT 4—TRANSFER OF PARASITIC LARVAE FROM LAMB TO LAMB AND FROM LAMB TO CALF: Four lambs were each inoculated with 100,000 infective larvae and killed 20 days later. As above, a mixture of L4 and adolescents was recovered. Of these, 3,400 and 6,500 worms were administered orally to each of two lambs, respectively, and 4,600 and 5,400

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Table 1. Numbers and stages of *Ostertagia ostertagi* recovered from lambs at various intervals after oral inoculation with 100,000 infective larvae.

Lamb. No.	Days to necropsy	Worms recovered					
		Number			Stages of development (%)		
		Contents	Digest	Total	L3	L4	Immature adult
366	22	6,000	34,100	40,100	52	33	15
554	26	4,000	66,000	70,000	40	40	20
209	30	6,700	21,600	28,300	13	87	0
655	35	300	8,400	8,700	2	98	0
709	40	300	6,600	6,900	2	98	0

to each of two calves, respectively. Fecal samples were collected daily for 20 days, when lambs and calves were killed.

Results

EXPERIMENT 1—ADMINISTRATION OF INFECTIVE LARVAE TO LAMBS: No patent infections were detected in any of the 17 lambs inoculated with infective larvae. There was no evidence of parasitic disease, even in the two lambs given half a million larvae. The five lambs killed from 22 to 40 days after inoculation (DAI) had from 2,300 to 70,000 *O. ostertagi*; from 76 to 94% of these worms were recovered from the digests (Table 1). Adolescent fifth-stage worms were not recovered after 30 days postinfection and the numbers of immature worms recovered between 26 and 40 days postinfection declined with time There was no evidence of edema, hyperemia, or fibrinonecrosis; however, there were scattered nodules about 5 mm wide and 2 to 3 mm high. These lesions were most evident in the lambs killed soonest after inoculation. Sections of excised lesions showed larvae situated in the gastric pits.

EXPERIMENT 2—TRANSFER OF ADULT WORMS FROM CALF TO LAMB: Eggs of *O. ostertagi* were present in feces passed by lambs 24 hr after the transfer of adult worms from calves. Egg counts as high as 800 per gram of feces were noted during the first few days, but EPG dropped gradually so that by day 84 it was only 26. The number of worms recovered at necropsy was: day 12—300; day 27—400; day 45—80; and day 84—7. These worms were all sexually mature adults.

EXPERIMENT 3—TRANSFER OF PARASITIC LARVAE FROM CALF TO LAMB: Of the worms transferred from the donor calves about 70% were L4 and the remainder were immature adults. The two lambs given the oral transfer of parasitic larvae became patent for *O. ostertagi* 6 and 7 days after transfer, respectively, and EPG reached peaks of 64 and 90. Two hundred seventy and 350 *O. ostertagi* normal sexually mature adults were recovered from them at necropsy 22 and 30 days after transfer. All females had a fully developed vulval flap distinctive of *O. ostertagi*.

EXPERIMENT 4—TRANSFER OF PARASITIC LARVAE FROM LAMB TO LAMB AND FROM LAMB TO CALF: Of the worms transferred from donor lambs, about 50% were L3, 40% L4, and 10% adolescent. No patent infections developed in either the lambs or calves given transfer inoculations, and no worms were recovered at necropsy 20 days after transfer.

Discussion

The results of this study confirm and extend the observations of Herlich (1958), Bessanov (1959), and Pandey (1971) that *O. ostertagi* does not reach sexual maturity in sheep inoculated with infective larvae. At the same time, this study shows conclusively that infective larvae can exsheath in lambs, enter the gastric pits, undergo development through the adolescent stage, and produce lesions similar to, although less extensive than, those observed in the bovine abomasum (Osborne, Batte, and Bell, 1960). However, the parasite is less pathogenic to sheep than to cattle as inoculation of lambs with 500,000 larvae produced no visible effects in this study, whereas 100,000 produced diarrhea and weight loss in calves (Ritchie et al., 1966) and 400,000 caused severe Type I ostertagiasis (Anderson et al., 1966).

Although sexual maturity is not attained in

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lambs inoculated with infective larvae, transfer infections with gravid adults or with mixtures of L3 to immature fifth stages did result in patent infections. These results show that there is nothing inherent to the lamb's abomasal milieu that prevents sexual maturation and long maintenance (at least 84 days in one lamb) of *O. ostertagi*. Some infective larvae managed to develop into adolescent adults by day 22, but all adolescents had been eliminated by day 30 and even L3 and L4 were much fewer by days 35 and 40; therefore, one can infer an immunological basis for the control of *O. ostertagi* in the abnormal host. The fact that transfer of L3 to immature fifth stages from lamb to lamb and calf failed to produce patent infections suggests that the inhibiting mechanism, whatever it may be, is irreversible.

The recurring reports of adult *O. ostertagi* recovered from sheep may be due to erroneous identifications and identification of adolescent stages. One cannot entirely preclude the possibility that in some instances under natural grazing conditions, small numbers of *O. ostertagi* may attain sexual maturity in sheep.

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Parasites of Greater Sandhill Cranes (*Grus canadensis tabida*) on Their Wintering Grounds in Florida[1]

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ABSTRACT: Twenty-two species of parasites were recovered from 74 greater sandhill cranes (*Grus canadensis tabida*) from four localities in Florida during 1970–73. These included three species of coccidia, one haemosporida, five trematodes, nine nematodes, and four biting lice. Twenty are new host records. Quantitative data are given only for helminth infections. The most common helminths were *Orchipedum jolliei*, *Strongyloides* sp., and *Tetrameres grusi*. Measurements of specimens of *O. jolliei* were smaller for flukes from multiple infections, indicating a possible "crowding effect." The numbers of helminth species per infected host averaged three (range, 1–4) with only one crane free of helminths. The total number of helminths per infected bird averaged 39 (range, 1–292). The most heavily parasitized organs were the small intestine and the proventriculus.

There are six subspecies of the sandhill crane (*Grus canadensis*) all of which occur only in the Western Hemisphere. Two of these, the greater sandhill crane (*G. c. tabida*) and the Florida sandhill crane (*G. c. pratensis*), occur in Florida (Fisher et al., 1969). Cranes that breed in Florida are *G. c. pratensis*, whereas the northern migrants, which mingle with the resident populations during the winter, are *G. c. tabida* (Williams and Phillips, 1972). Both birds are listed as rare on the U. S. Fish and Wildlife Service's "rare and endangered species" list (Committee on Rare and Endangered Fish and Wildlife of the United States, 1966).

The present report is based on the coccidia, haemosporida, helminths, and mallophagans collected from greater sandhill cranes obtained from four localities in Florida.

Existing records of parasites from greater sandhill cranes include one trematode (*Brachylecithum gruis* Denton and Byrd, 1951) and two biting lice [*Esthiopterum brevicephalum* (McGregor, 1917) and *Gruimenopon canadense* Edwards, 1949].

Materials and Methods

From February 1970 through February 1973, 74 sandhill cranes were examined for parasites. The birds were from wintering flocks on: (1) Paynes Prairie, Alachua County (46 birds), (2) Juniper Prairie, Lake County (1 bird), (3) Lake X, Osceola County (2 birds), and (4) KD Ranch, Highlands County (25 birds). All were yearlings or older individuals from populations known to be predominantly greater sandhill cranes.

Thirty-four of the birds were obtained as a result of mortality due to trapping operations being conducted by the Florida Game and Fresh Water Fish Commission (Williams and Phillips, 1973) and were examined at necropsy. Some were examined within a few hours after death, but most were frozen and examined later. Procedures for recovery, killing, fixing, and studying helminths were similar to those described by Kinsella and Forrester (1972).

Blood samples were obtained from 51 cranes, five of which were from the 34 birds examined at necropsy, and 46 were from birds captured and later released alive. Films were made with blood taken from a wing vein and stained with Giemsa's stain. To detect blood protozoans approximately 30,000 red blood cells were examined on each slide using oil immersion (1,000×).

Fecal samples were obtained from 46 cranes, 16 of which were from birds examined at necropsy. The remaining 30 were from live-trapped birds which were later released. These samples were placed in 2% potassium dichromate solution and later examined for coccidian oocysts using standard flotation techniques.

Ectoparasites were collected with forceps or a fine-tipped brush, killed, fixed, and preserved in 70% ethyl alcohol, and later cleared and mounted following standard techniques.

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Table 1. Helminths of 34 greater sandhill cranes collected in Florida from February 1970 to February 1973.

Helminth	No. birds infected	No. worms/infection	
		Mean	(Range)
Trematoda			
Orchipedum jolliei (8, 9)*	19	7	(1–28)
Brachylaima fuscatum (4)	5	3	(1–5)
Strigea gruis (3,4)	2	15	(11–19)
Prosthogonimus macrorchis (6)	1	1	–
Echinostomidae (5)	1	1	–
Nematoda			
Tetrameres grusi (1)	23	22	(1–79)
Strongyloides sp. (3, 4, 5)	17	31	(1–188)
Spiruroid larvae† (1, 2)	10	11	(1–31)
Syngamus trachea (8)	7	3	(1–4)
Trichostrongylus tenuis (3)	1	6	–
Dispharynx nasuta (1)	1	2	–
Synhimantus sp. (2)	1	1	–
Amidostomum sp. (2)	1	1	–
Capillaria sp. (4)	1	1	–
Chandlerella sp. (7)	1	1	–

* Numbers in parentheses indicate location in host: (1) proventriculus, (2) under gizzard lining, (3) duodenum, (4) lower small intestine, (5) ceca, (6) cloaca, (7) heart, (8) trachea, (9) lungs.
† Probably larvae of *Tetrameres grusi*.

Results and Discussion

Twenty-two species of parasites were recovered including three coccidia, one haemosporida, five trematodes, nine nematodes, and four biting lice. All except two are new host records.

Protozoa

Of the 51 blood films examined, four contained light infections of a species of *Haemoproteus*. This species, morphologically similar to *H. antigone* de Mello, 1935, is being studied further. Representative blood films have been deposited in the collection of the WHO International Reference Centre for Avian Malaria Parasites at Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

Oocysts of three undescribed species of *Eimeria* were found commonly in feces from cranes collected in all localities. These will be described and discussed further elsewhere.

Helminths

Table 1 gives: (1) the species of helminths encountered, (2) the site of infection for each helminth, (3) the number of hosts infected with each helminth, and (4) mean numbers (and ranges) of worms per infection. The number of helminth species per infected host varied from 1 to 4 (mean, 3) with only one crane free of helminths. The total number of helminths per infected bird ranged from 1 to 292 (mean, 39). The small intestine was the most heavily parasitized organ and contained an average of 17 parasites (representing five species) per bird. Within the small intestine the most heavily parasitized section was the lower small intestine which contained an average of nine worms (of four species) per bird compared to the duodenum which contained only six worms (of three species) per bird. The second most heavily parasitized organ was the proventriculus which averaged 15 parasites per bird. The majority of the parasites in the proventriculus were *Tetrameres grusi* Shumakovich, 1946. Additional information on the numbers of species and burdens in each organ is presented in Table 2.

Table 2. Numbers of species, prevalence of infection, and burdens of helminths in organs of 34 greater sandhill cranes from Florida.

Organ	No. birds infected	Total no. helminth species	No. helminths	
			Mean	(Range)
Small intestine	28	5	17	(1–167)
Proventriculus	23	2	15	(1–79)
Trachea	24	2	4	(1–27)
Gizzard	9	3	3	(1–31)
Lungs	3	1	1	–
Ceca	1	1	1	–
Cloaca	1	1	1	–
Heart	1	1	1	–
All organs	–	14	39	(1–292)

Trematoda

The most common trematode encountered was *Orchipedum jolliei* which was described by Schell (1967) on the basis of one specimen from the trachea of a lesser sandhill crane, *G. c. canadensis*, from Idaho. It has been found again in one of 12 lesser sandhill cranes in West Texas (Burnham, 1972). Ours is the first report of this fluke in greater sandhill cranes. In Table 3 measurements are given for 20 specimens and these are compared to measurements of the type specimen of Schell (1967). Measurements are also given in Table 3 of specimens from infections in which only one or two worms were found in comparison to in-

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Table 3. Comparative measurements of *Orchipedum jolliei* from greater sandhill cranes in Florida.

Measurement*	Schell's (1967) specimen	Specimens from present study: Infections with 1 or 2 worms/host Mean	(Range)	Infections with 8–28 worms/host Mean	(Range)	All specimens Mean	(Range)
Body length (mm)	19.0	21.4	(16.0–24.3)	18.7	(16.2–21.1)	19.7	(16.0–24.3)
Body width (mm)	2.7	2.7	(2.4–3.1)	2.3	(1.8–2.7)	2.5	(1.8–3.1)
Length of oral sucker (mm)	1.2	1.2	(1.1–1.2)	1.0	(0.9–1.1)	1.1	(0.9–1.2)
Width of oral sucker (mm)	1.4	1.3	(1.2–1.4)	1.2	(1.0–1.3)	1.2	(1.0–1.4)
Diameter of ventral sucker (mm)	1.6	1.6	(1.5–1.7)	1.4	(1.1–1.6)	1.5	(1.1–1.7)
Length of pharynx	733	682	(644–714)	649	(532–700)	662	(532–714)
Width of pharynx	530	588	(546–630)	520	(434–574)	547	(434–630)
Number of testes	300	306	(235–400)	310	(235–425)	309	(235–425)
Length of ovary	764	597	(448–728)	519	(350–644)	550	(350–728)
Width of ovary	514	494	(406–574)	394	(210–462)	434	(210–574)
Egg length	92	74	(62–90)	72	(65–85)	73	(62–90)
Egg width	61	46	(39–55)	45	(37–57)	46	(37–57)
Transverse ridge length (mm)	—	9.7	(5.0–11.2)	9.4	(7.5–11.1)	9.5	(5.0–11.2)
% transverse ridges	33	45	(31–51)	51	(44–56)	48	(31–56)
No. specimens measured	1	8		12		20	

* Measurements are in microns unless otherwise indicated.

fections with eight to 28 worms per host. In all cases flukes from multiple infections were smaller, indicating a possible "crowding effect."

In some infections *Orchipedum* was associated with an excess of mucus and some inflammation was seen in the trachea near the site of worm attachment.

Brachylaima fuscatum (Rudolphi, 1819) is a common trematode of birds which has been reported from a variety of passeriform and galliform birds (Yamaguti, 1971). This is the first report from a gruiform.

Strigea gruis was described by Dubois and Rausch (1964) from specimens obtained from three lesser sandhill cranes in Alaska. It has not been reported from any other host.

The presence of *Prosthogonimus macrorchis* Macy, 1934, is unexplained, particularly since other hosts [Florida ducks, *Anas platyrhynchos fulvigula* (Kinsella and Forrester, 1972) and wild turkeys, *Meleagris gallopavo osceola* (Hon, 1973)] in the same regions are infected with *P. ovatus* (Rudolphi, 1803).

A single specimen of the family Echinostomidae, morphologically similar to *Echinostoma*, was recovered from the cecum of one crane. Further identification was not possible since the specimen was obtained from a bird that had been frozen and the collar spines were missing.

Nematoda

The most common nematode was *Tetrameres grusi*, originally described on the basis of five male specimens taken from the gray crane (*Grus grus*) in western Siberia (Shumakovich, 1946). This species in sandhill cranes has been discussed in another paper (Bush, Pence, and Forrester, 1973) in which the male was redescribed and the female described for the first time. This represents the first report of this species from North America. Burnham (1972) reported infections of an unidentified species of *Tropisurus* in 20 of 57 lesser sandhill cranes in Texas, which may have been *T. grusi*. In the present study spiruroid larvae, probably of *T. grusi*, were found in the proventriculus and under the gizzard lining of 10 cranes.

The second most common nematode, *Strongyloides* sp., was found in 17 cranes and apparently represents an undescribed species. Parasitic females were 2.50–3.25 mm (mean, 2.87 mm) in total length and contained up to six eggs (mean, three eggs). Esophagus length was 615–840 μ (mean, 708 μ). Free-living males and females were cultured from crane feces. Males were 588–868 μ (751 μ) in total length with spicules 34–40 μ (37 μ) and esophagus 85–135 μ (115 μ) in length. Free-living females were 1.11–1.19 mm (1.15 mm) long with esophagus 165–175 μ (170 μ) in length. Measurements of free-living males and parasitic females were close to those published by Cram (1929) for *S. avium* from chickens but free-living females of the species of *Strongyloides* from cranes were considerably larger than those of *S. avium*.

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Syngamus trachea (Montagu, 1811) was the third most prevalent nematode, but occurred in small numbers. This cosmopolitan species is fairly common in Galliformes and Passeriformes and has been reported from six other orders of birds (Yamaguti, 1961). This is the first report of it in a gruiform.

One male specimen of an undescribed species of *Synhimantus* was recovered from a crane from the KD Ranch in south Florida. The same species has been recovered also from wild turkeys and white ibis, *Eudocimus albus*, from Florida (Hon, 1973; Bush, 1973). The specimen was 5.2 mm long, the long spicule was 628 μ, the short spicule was 148 μ, and the cordons extended posteriorly 112 μ from the anterior end.

The remaining six nematodes were each represented by a single infection and all are probably abnormal parasites for this host. Two, *Trichostrongylus tenuis* (Mehlis, 1846) and *Dispharynx nasuta* (Rudolphi, 1819), are common in other hosts collected in the same general areas (Hon, 1973). These two species have wide host ranges and may represent seasonally abnormal parasites which occur in relative paucity in cranes on their wintering grounds.

Amidostomum sp., *Capillaria* sp., and *Chandlerella* sp. were each represented by a single individual and for this reason specific determinations were not made.

Mallophaga

Four species of biting lice were found: *Esthiopterum brevicephalum*, *Gruimenopon canadense*, *Saemundssonia sagulata* Timmermann, 1971, and *Heleonomus assimilis* (Pioget, 1880). *Esthiopterum brevicephalum* has been reported from greater sandhill cranes in Minnesota (McGregor, 1917) and *G. canadense* from lesser sandhill cranes in Alaska and Texas (Edwards, 1949) and greater sandhill cranes in Indiana (Carriker, 1958). *Saemundssonia sagulata* has been reported only from *Grus c. pratensis* which is its type host (Timmermann, 1971). This is the first report of *H. assimilis* from the greater sandhill crane, however, which was previously known only from the whooping crane, *Grus americana* (Emerson, 1964).

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The Influence of Intermediate Hosts on the Infection Pattern of *Protospirura numidica criceticola* Quentin, Karimi, and Rodriguez De Almeida, 1968 (Nematoda: Spiruridae) in the Bonneville Basin, Utah

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ABSTRACT: The infection pattern of *Protospirura numidica criceticola* Quentin, Karimi, and Rodriguez De Almeida, in the Bonneville Basin, Utah, is reported. The white-footed deer mouse, *Peromyscus maniculatus sonoriensis* (LeConte), was selected as the experimental host because it is a natural host in this region. Incidence of infection in the desert habitats was highest during late summer and autumn but declined in early winter becoming low by spring. Mountain habitats showed a much lower uniform yearly infection rate. Three new insect intermediate hosts were revealed. These include the grasshoppers, *Melanoplus femur-rubrum* (DeGeer) and *M. atlanis* (Riley), and the cricket, *Gryllus pennsylvanicus* (Burm.). A darkling beetle, *Eleodes tuberculata patruelis* Blaisdell, was the only proved insect host previously known from this area. Nematode eggs were viable in excess of 3 years. Larval development at 25 C occurred at different rates in different species of insects. Infectivity of encysted juveniles in *E. t. patruelis* did not decrease up to 170 days postinfection. Newly hatched larvae entered a quiescent state in the hemocoel of this beetle at 5 C but later resumed normal development at 25 C. Deer mice harbored adult worms in the stomach and lower esophagus up to 130 days postinfection.

Protospirura numidica criceticola is widespread, occurring in all major habitats from vegetated dunes of the Great Salt Lake Desert at 4,200 ft to alpine tundra at 11,500 ft. In the Bonneville Basin of western Utah at least seven rodent species are parasitized. They include the common deer mice, *Peromyscus maniculatus sonoriensis* (LeConte) and *P. m. rufinus* (Merriam), pinion mouse, *P. truei nevadensis* Hall and Hoffmeister, canyon mouse, *P. crinitus pergracilis* Goldman, western harvest mouse, *Reithrodontomys megalotis megalotis* (Baird), grasshopper mouse, *Onychomys leucogaster utahensis* Goldman, least chipmunk, *Eutamias minimus pictus* (Allen),

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and two subspecies of the Ord kangaroo rat, *Dipodomys ordii celeripes* Durrant and *D. o. marshalli* Goldman.

Seurat (1914) established the genus *Protospirura* with *P. numidica* from the stomach of *Felis ocreata* Bate as the type species. Years later, Quentin, Karimi, and Rodriguez De Almeida (1968) collected a spiruride from *Zygodontomys lasiurus* (Lund) in Brazil and considered it to be a distinct subspecies based on geographical and ecological differences and a larger spicule in the male. Consequently, the name *P. numidica criceticola* n. subsp. was proposed and includes all the North American helminths previously named *Protospirura numidica*.

A study was made during the course of 1 year to determine why yearly infection patterns for this nematode are seasonal in the desert habitats of the Bonneville Basin as opposed to a low steady rate in the mountains. The white-footed deer mouse, *P. m. sonoriensis*, was selected as the experimental host because it is a natural host in this region and is ubiquitous throughout western Utah (Durrant, 1952). A description of the study area is given by Fautin (1946) and Vest (1962).

Crook and Grundmann (1964) first elucidated the life cycle of *P. n. criceticola* in the Bonneville Basin by demonstrating the darkling beetle, *Eleodes tuberculata patruelis* Blaisdell, as the natural intermediate host. Dyer and Olsen (1967) experimentally infected the cricket, *Acheta domestica* (Linn.), beetle, *E. obsoleta* (Say), and grasshopper, *Melanoplus femur-rubrum* (DeGeer). Natural infections were found in the latter two species. Quentin, Karimi, and Rodriguez De Almeida (1968) established laboratory infections in the linear earwig, *Doru lineare* Escherich, and cockroach, *Periplaneta americana* (Linn.).

Materials and Methods

Arthropods tested as intermediate hosts were collected in and around rodent burrows in the same locality from which *P. n. criceticola* was colonized. Included were darkling beetles, *E. t. patruelis* and *E. hispilabris sculptilis* Blaisdell, grasshoppers, *M. femur-rubrum* and *M. atlanis* (Riley), and a black field cricket, *Gryllus pennsylvanicus* (Burm.). A butterfly net was used to collect grasshoppers while beetles and crickets were gathered by hand.

No attempt was made to rear insects in the laboratory. Specimens collected were placed in aluminum insect cages prepared to simulate natural surroundings. Ambient laboratory conditions of 25 C and approximately 35% relative humidity were maintained. Grasshoppers were fed freshly grown barley, oats, wheat, corn, and sunflower seedlings. Darkling beetles were given a mixture of lettuce and horse manure while crickets were provided with grass, lettuce, and powdered "Purina Mouse Chow." Standard chick waterers were placed in all cages.

Ovigerous female worms containing infective ova were removed from the stomachs of snap-trapped *P. m. sonoriensis*. Test groups of 15 insects became infected by ingesting embryonated eggs mixed with either deer mouse feces or apple pulp. A similar number was examined for natural infections. Anesthetized insects were pinned to the bottoms of wax-poured petri dishes, dissected in tap water, and examined for the presence of encysted juveniles. Laboratory-reared, parasite-free deer mice were given nematode cysts via stomach tube and later necropsied. The esophagus, stomach, and small intestine were opened in physiological saline to free the worms, the majority of which were readily found upon gross examination. Parasites were preserved in 4% formalin.

Results

Experimental infections were obtained in all insect species tested with the exception of *E. h. sculptilis*. No natural infections were found (Table 1). Since it had not been determined which insects were coprophagic, separate groups of individual species were exposed to either deer mouse feces or apple pulp, each containing various numbers of nematode eggs. A standard dose was not used as it was impossible to feed each insect an equal amount. All test insects readily consumed the apple pulp. Grasshoppers and beetles ingested deer mouse feces but crickets would not, even after periods of starvation.

An experiment was conducted to determine the rate at which nematode larvae would develop in the different insect species. Members

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Table 1. Results of feeding deer mouse feces and apple pulp containing eggs of *Protospirura numidica criceticola* to darkling beetles, *Eleodes tuberculata patruelis* and *E. hispilabris sculptilis*, grasshoppers, *Melanoplus femur-rubrum* and *M. atlanis*, and the cricket, *Gryllus pennsylvanicus*.

Insects	No. examined	No. infected	No. of cysts recovered	
			Mean	Range
E. tuberculata patruelis				
A	15	7	1.7	1–2
B	15	13	6.2	1–16
C	15	0	—	—
E. hispilabris sculptilis				
A	15	0	—	—
B	15	0	—	—
C	15	0	—	—
M. femur-rubrum				
A	15	13	6.2	1–12
B	15	14	18.4	4–38
C	15	0	—	—
M. atlanis				
A	12	9	62.0	10–170
B	15	14	8.9	3–15
C	15	0	—	—
G. pennsylvanicus				
A	15	3	1	1
B	15	15	61.0	21–96
C	15	0	—	—

A = Fed eggs in deer mouse feces.
B = Fed eggs in apple pulp.
C = Control.

of each species were divided into separate groups and exposed to infective ova. Individuals from each group were killed at 5-day intervals, commencing 20 days postinfection and continuing to day 30, then at 2-day intervals until day 44. Nematode cysts recovered were administered to deer mice which were necropsied 20 days later. Results showed that *M. atlanis* and *G. pennsylvanicus* contained infective third-stage juveniles 30 days after ingesting eggs whereas 36 days were required for *M. femur-rubrum* and *E. t. patruelis* (Table 2). Larval cysts containing one to five worms were recovered from muscles and malpighian tubules between 20 and 30 days postinfection. After 40 days, the majority of cysts were free in the hemocoel, particularly congested in the prothorax immediately behind the head.

To establish the length of time that *P. m. sonoriensis* harbored infections, an experiment was conducted wherein 32 deer mice were evenly divided into four groups and each mouse given 18 cysts. The first group was killed after 40 days and examined for the presence of worms. The remaining three groups were dissected on days 70, 100, and 130, respectively. The number of adult worms recovered ranged from 1 to 22. No significant decline in worms recovered was noted between 40 and 130 days (Table 3). The lower esophagus and stomach were often irregularly distended due to heavy worm burdens.

Table 2. Period required for third-stage juveniles of *Protospirura numidica criceticola* to become infective in cysts from the darkling beetle, *Eleodes tuberculata patruelis*, grasshoppers, *Melanoplus femur-rubrum* and *M. atlanis*, and the cricket, *Gryllus pennsylvanicus*.

Insects	Age of encysted juveniles (days)									
	20	25	30	32	34	36	38	40	42	44
E. tuberculata patruelis										
A	6	7	15	12	14	8	14	9	11	19
B	0	0	0	0	0	3	8	4	0	10
M. femur-rubrum										
A	10	8	16	12	9	17	13	23	14	10
B	0	0	0	0	0	4	5	16	0	3
M. atlanis										
A	10	8	6	24	18	14	25	21	15	—
B	0	0	2	15	11	10	14	17	9	—
G. pennsylvanicus										
A	12	11	19	33	23	13	9	8	14	15
B	0	0	10	25	20	2	0	8	14	14

A = Number of encysted juveniles given/mouse.
B = Number of worms recovered/mouse after 20 days.

To determine the duration that juvenile worms remain infectious in the hemocoel of insects, a group of 25 beetles were infected. A single beetle was killed at 5-day intervals between 50 and 170 days postinfection. All cysts recovered were given to deer mice which were necropsied 20 days later. Results showed that infectivity of encysted juveniles did not decrease with age. Cysts recovered from *E. t. patruelis* 170 days postinfection produced infection rates in deer mice comparable to those removed at 50 days (Fig. 1). The number of encysted juveniles given to each mouse ranged from five to 26 as based on the number of cysts recovered from individual beetles.

The effects of hibernation on experimentally infected beetles were investigated. Forty *E. t. patruelis* were exposed to embryonated eggs and placed in containers filled with moistened soil, grass, and horse manure. Of this group, 30 beetles were refrigerated at 11 C for 9 days and then stored at 5 C. The remaining 10 beetles were retained as controls at room temperature. Dissection of the control beetles at 2-day intervals commencing 20 days postinfection revealed normal parasite development. After 84 days at 5 C, all refrigerated beetles were returned to room temperature.

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Table 3. Results of infecting 32 deer mice with approximately 18 cysts/mouse. Groups A, B, C, and D were dissected on days 40, 70, 100, and 130, respectively.

Group	No. examined	No. infected	No. of male worms	No. of female worms	No. of adult worms recovered	
					Mean	Range
A	8	8	51	70	15.1	1–22
B	8	8	41	56	12.1	6–21
C	8	8	48	56	13.0	7–16
D	8	8	36	64	12.5	8–18

Nine beetles were immediately killed and examined under a dissecting scope. No parasite cysts were noted. However, cysts were recovered from the remaining 21 beetles which were dissected at 5-day intervals, beginning on day 20 posthibernation and continuing to day 30, then at 3-day intervals until day 42. This suggested that nematode larvae were inhibited in development while the host was dormant but were capable of resuming growth when suitable conditions were again restored.

Discussion

Protospirura numidica criceticola shows a seasonal occurrence in deer mouse populations in desert habitats of the Bonneville Basin. Infection rates are low in March, April, May, and early June, rise rapidly in late June, July, August, and September, and begin to taper off in early winter. Grundmann (1957) reported infection by this nematode in desert localities to approach 100% by late September. This seasonal expression would indicate that the parasite has a period of residence in the definitive host of less than 1 year.

Population density of deer mice is greater in late summer and autumn resulting in a more limited home range. Grasshoppers such as *M. femur-rubrum* and *M. atlanis* do not emerge as nymphs until late May and subsequently attain their highest numbers during the late summer season. When these two conditions are synchronized, an increased infection rate is, in fact, expected.

Deer mice select food on the basis of seasonal abundance as indicated by analysis of stomach contents (Warnock and Grundmann, 1963). Spring and early summer diets contain large amounts of foliage. Seeds become a major component as fruits begin to ripen. As

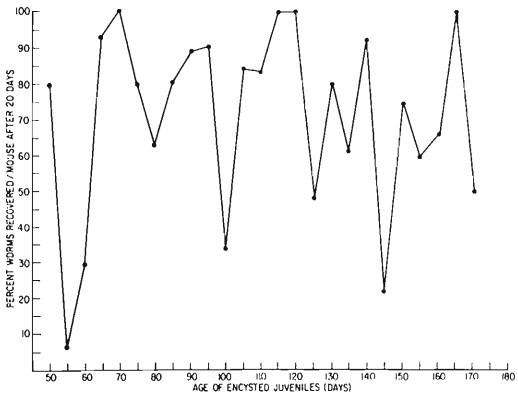


Figure 1. Duration of juvenile infectivity in *Eleodes tuberculata patruelis*.

the dry summer season advances considerable quantities of insects and fecal pellets, both their own and from other species, are consumed. Grasshoppers, beetles, and crickets serving as intermediate hosts for this parasite attain their greatest impact on diet during this period. Derrick (1971) found this dietary shift to occur to a lesser extent in mountain habitats of the region because adequate plant food remains abundant. He reported infection by *P. n. criceticola* in rodents to persist at a low level throughout the year and never exceed 15.2% in any one month. Consequently, a seasonal infection pattern usually does not occur at higher elevations as it does in the more xeric habitats of the Basin.

The majority of insects become infected in nature by eating contaminated feces. Darkling beetles are naturally coprophagic and grasshoppers have been observed scavenging on dried cow manure (Lavigne and Pfadt, 1964). Since crickets are not coprophagic and assuming they serve as a natural intermediate host for this parasite, it seems likely that they become infected in nature by eating contaminated vegetation rather than fecal matter. Once infected, the insects serve as a natural parasite reservoir for neighboring deer mice.

Grundmann and Warnock (1964) reported nematode eggs to remain viable in excess of 3 years. Larval worms develop to maturity in the hemocoel of suitable insects within 36 days and remain infectious for several months more. Insects becoming parasitized in early spring are

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thus capable of infecting deer mice throughout the summer. By autumn and early winter, contact between deer mice and insects declines. Indices of parasitism likewise decline so that by late March few infected hosts can be found. Survival of the nematode in a locality must bridge gaps when reproducing adult worms are absent in definitive hosts. *M. femur-rubrum* and *M. atlanis* overwinter in the egg stage and do not retain the parasite. However, *G. pennsylvanicus* and *E. t. patruelis* hibernate overwinter as nymphs and adults and may harbor infections. These two species of insects when infected in late summer could serve as a source of immediate infection the following spring. Deer mice feeding on these parasitized insects then become infected with worms reaching maturity in their stomachs in about 36 days (Crook and Grundmann, 1964). These mice may harbor the parasite for months, continually seeding their domain with infective ova.

Yearly infection patterns for *P. n. criceticola* are seasonal in the desert habitats of the Bonneville Basin as opposed to a low steady rate in the mountains. High summer and autumn infection rates in deer mouse populations in desert habitats have been attributed to (1) seasonal occurrence of the parasite as based on the length of residence in the definitive host being less than 1 year, (2) the increased density of deer mouse populations forcing a more limited home range, and (3) the increased use of insects as food as desert vegetation becomes less abundant. Indices of parasitism decline in early winter becoming low by spring. Survival in a locality is dependent on (1) resistance and viability of nematode eggs for several years, (2) rate and duration of larval infectivity in insect intermediate hosts, (3) larval ability to enter into a quiescent state in the hemocoel of insects during hibernation, and (4) prolonged retention of adult worms by some deer mice.

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Massive Leech Infesation on a White Catfish (*Ictalurus catus*): A Histopathological Consideration[1]

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ABSTRACT: A massive infestation by the leech, *Cystobranchus virginicus* Hoffman, 1964, on a white catfish, *Ictalurus catus*, captured in the York River, Virginia, is described. The histopathological changes in the dermis and epidermis at the attachment sites of the leeches included extensive granulation, focal epithelioma, and displacement of the epithelial and dermal layers with subsequent proliferation.

Infestations of leeches on catfishes are known to occur in the southeastern United States (Rogers, 1971) but no descriptions of histopathology resulting from the action of leeches are available. We shall describe an infestation on white catfish from the York River, Virginia, from a histopathological viewpoint.

Materials and Methods

In the aftermath of tropical storm Agnes a large white catfish (*Ictalurus catus*) was observed floating near our laboratory pier in the lower York River, Virginia, on 28 June 1972. It measured 338 mm long and weighed 602 gm. Salinity was 8 ppt at the time of capture. The average salinity at Gloucester Point is approximately 19 ppt for late June (Data Bank, Virginia Institute of Marine Science). The fish was alive when captured but was in distress and was heavily infested with leeches.

Leech attachment sites on the fish were excised and fixed in 10% formol-saline. Histological (7–10 μm) sections were prepared from paraffin-embedded tissue and stained with Harris' hematoxylin–eosin.

Description of the infestation

Over 500 leeches were found attached to the body of the fish. They were concentrated on the sides of the mouth, along the gill aperture under the operculum, in the skin fold behind the lower jaw, and at the bases of the fins.

The general area of the attachment sites of the leeches was densely dotted with red foci, which were particularly distinct on the white fish skin. In the areas supporting the heaviest population the foci became confluent, forming large, red, intensely irritated sections (Figs. 1, 2).

A sample of the leeches was sent to Prof. Marvin C. Meyer, who identified them as *Cystobranchus virginicus* Hoffman, 1964, previously reported from the Roanoke River near Shawsville, Montgomery County, Virginia. No host record data were available in that report.

Histopathology

Histopathological changes in the skin at the attachment sites included extensive inflammatory response, displacement and erosion of the dermal layers, and hyperplasia of the epithelium. Extensive cellular infiltration occurred in the epidermis, in the collagen layer of the dermis, and even in some areas of the subcutis and underlying muscles. It was especially evident in the opercular area and bases of the fins (Figs. 3, 4). Lymphatic spaces in the stratum germinativum increased in number. Focal and widely spread hemorrhages were present beneath the epithelium in the upper dermal layer.

The epithelial surface was frequently intact, but at some sites erosion of the epidermis was extensive and disruptive, affecting the

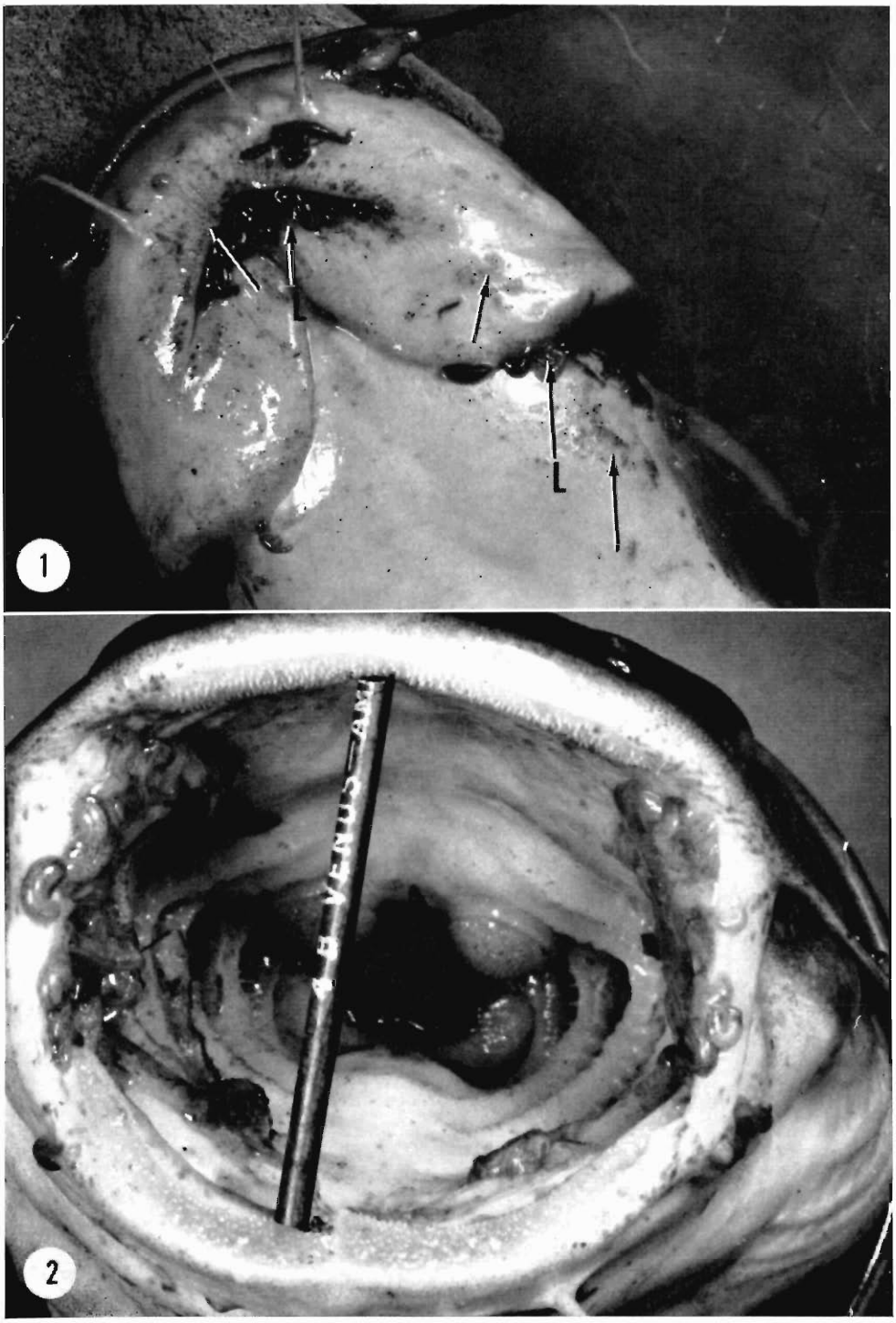
[1] Contribution No. 557 from the Virginia Institute of Marine Science, Gloucester Point, Virginia. This research was accomplished while the senior author was an NSF Senior Foreign Scientist Fellow in the School of Marine Science of the College of William and Mary.

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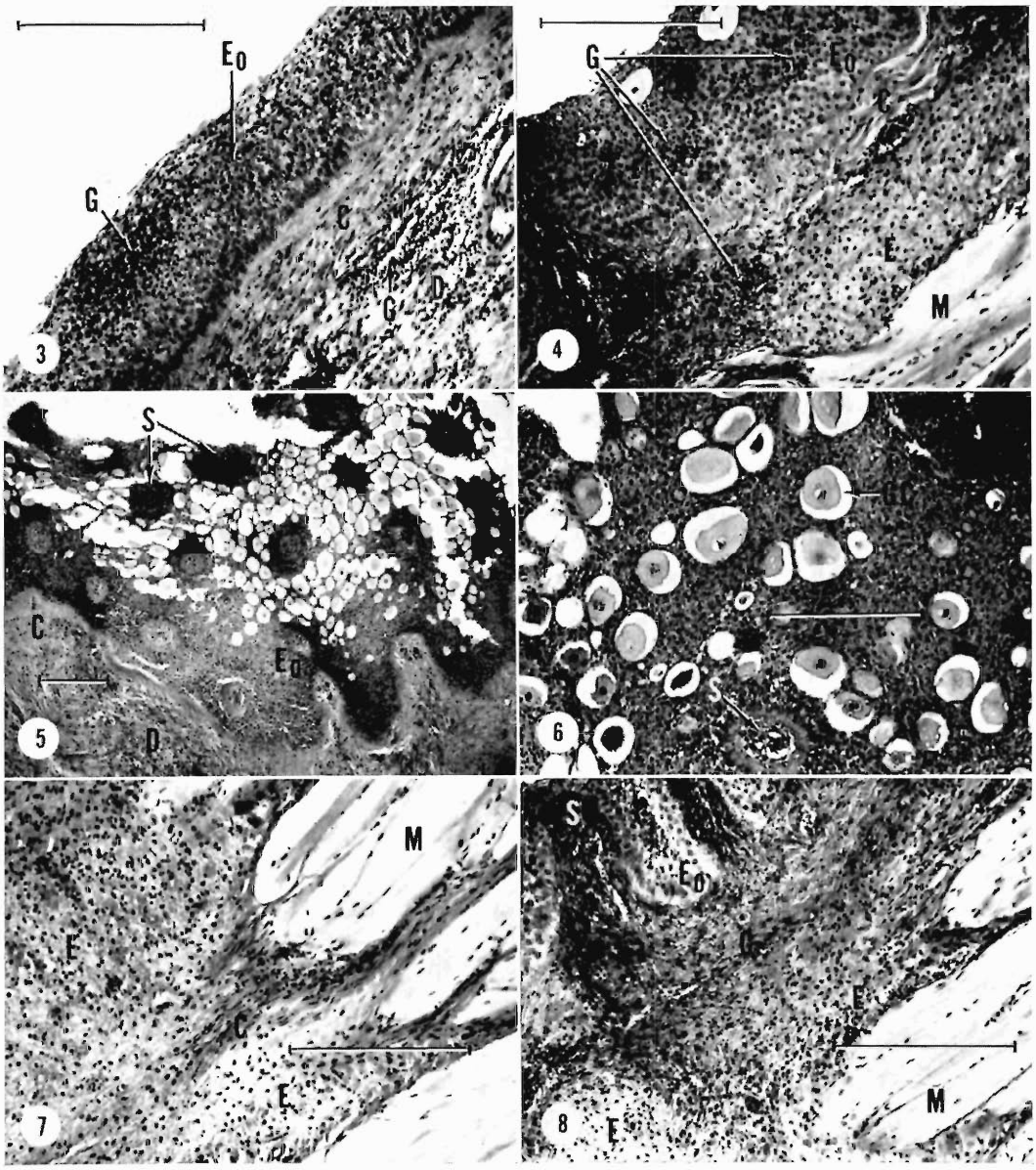
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Figures 1–2. Leech infestation of white catfish. 1. Ventral view of head of host showing red foci (arrows) and attachment sites of leeches (L). 2. Open mouth of host showing concentrations of leeches.

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Figures 3–8. Histological sections of skin. Scale bars for Figures 3–8 = 0.2 mm. Abbreviations: C, dermal collagen; D, subdermis; E, displaced epithelium; E°, surface epithelial layer; G, granulation sites; GC, club-shaped gland cells; M, muscle layer; S, connective tissue stromata in the epithelium. 3. Cross section showing areas of granulation. 4. Cross section showing additional areas of granulation and displacement of epithelium into the subdermis. 5. Oblique section showing proliferation of epithelium and club-shaped gland cells; note eroded surface layer. 6. Enlargement of area similar to Figure 5 showing detail of club-shaped gland cells. 7. Cross section showing displacement of epithelium into muscle layer. 8. Same section as above showing involvement in the deeper tissues.

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squamous and middle layers. The dermal collagen layer was disoriented in many places.

Massive papillomalike hyperplasia of both the giant skin gland cells ("club-shaped glands" of Mittal and Munshi, 1971) and the epithelium was evident on the surface of at least two sites examined from the ventral skin folds of the lower jaws. An expanded network of connective tissue stromata proliferated the hyperplastic epidermis and the surface of the epidermis was very eroded in this area (Figs. 5, 6).

In some areas the epithelium, together with strands of dermal collagen and bundles of fibroblasts, was pushed into subdermal layers, sometimes even into the muscular layer. This sunken epithelium proliferated extensively and penetrated the adjacent tissues (Figs. 4, 7, 8).

Discussion

Leeches of the genus *Cystobranchus* have been reported to frequently attack channel catfish stocks in southeastern United States fish ponds and cause problems (Rogers, 1971). The leech *C. virginicus*, involved in the infestation reported by us, appears to be also potentially destructive to catfish. This leech infestation probably originated in the upper reaches of the York River which is apparently the normal habitat for both fish and parasite. The white catfish is known to survive in estuarine water of 11 ppt, however, and occasionally may be found in more saline environs (Musick, J., Virginia Institute of Marine Science, pers. comm.). Since no other pathological changes in addition to those caused by the leeches were observed, it appears to us that the leeches were at least a major contributing factor to the distressed condition of the fish.

The histopathology of the leech infestation was interesting since it simulates neoplastic histological changes. The hyperplasia of the club-shaped gland cells of the epithelium mimics an epithelioma. Moreover, the displacement of the epithelium (apparently by mechanical action of the leech) into the dermis, subdermis, and musculature, and its subsequent proliferation there suggested a malignant tumor. In a similar manner, the dermal layer appeared to be pushed deeper into the musculature where it too proliferated. Spontaneous epithelioma on *Ictalurus* usually involved in hyperplasia of the epithelial cells rather than the gland cells (Harshbarger, 1972). However, a papilloma with proliferation of gland cells was seen in the African electric catfish (Paperna, unpublished data). Harshbarger (1972) also described cases of transition from papilloma to carcinoma in catfishes. Thus, in some cases ectoparasitic infestations on fish may induce extreme histological processes which are very similar in appearance to skin neoplasia.

Acknowledgments

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Development and Pathogenesis of a Root-Knot Nematode, *Meloidogyne javanica*

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ABSTRACT: Histological studies showed that the soybean variety N. C. Hampton was a favorable host for the root-knot nematode, *Meloidogyne javanica.* Infective second-stage larvae invaded young roots within 24 hr after inoculation. Most abundant penetration of the host occurred in the meristematic tissues, but the regions of cell enlargement and differentiation also were invaded; as many as 30 larvae were observed feeding on a single root tip. Larval penetration was both intercellular and intracellular and passage of larvae through the cortex caused distortion and necrosis of nearby cells. Nematodes were found feeding in cortex, endodermis, pericycle, and stele and this feeding resulted in hypertrophy, hyperplasia, and giant cell formation in tissues immediately surrounding the head of the nematode. Giant cells were associated with the development of *M. javanica* and were more abundant in the stelar tissue than in the cortex. Nematode females oviposited 30 days after inoculation and the life cycle was completed in about 35 days.

Root-knot nematodes are noted for their ability to incite marked morphological and anatomical changes in host roots (Bird, 1972; Dropkin, 1959; Dropkin and Nelson, 1960; Endo, 1971; Ibrahim et al., 1972). Dropkin (1959) demonstrated that host–parasite interactions between soybean varieties and *Meloidogyne* spp. could be used as bioassay procedures to distinguish races of root-knot nematodes. Dropkin and Nelson (1960) reported that giant cells formed by these nematodes on soybeans could be arranged into four morphological types depending on the host–parasite interactions.

The life cycle of *Meloidogyne* spp. has been the subject of numerous investigations. Tarjan (1952) studied four species of *Meloidogyne* infecting snapdragon roots and found no basic differences between the nematode species in regard to their development. However, life cycles of root-knot nematodes have manifested host effects based on species (Godfrey and Oliveira, 1932; Tarjan, 1952), nutrition (Bird, 1970; Oteifa, 1953), and ambient temperature (Bird and Wallace, 1965; Bryant and Wyllie, 1968; Dropkin, 1963).

The purposes of the present study were to describe the development of *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, in the roots of the soybean variety N. C. Hampton and the histological changes which occur in the infected root tissues.

Materials and Methods

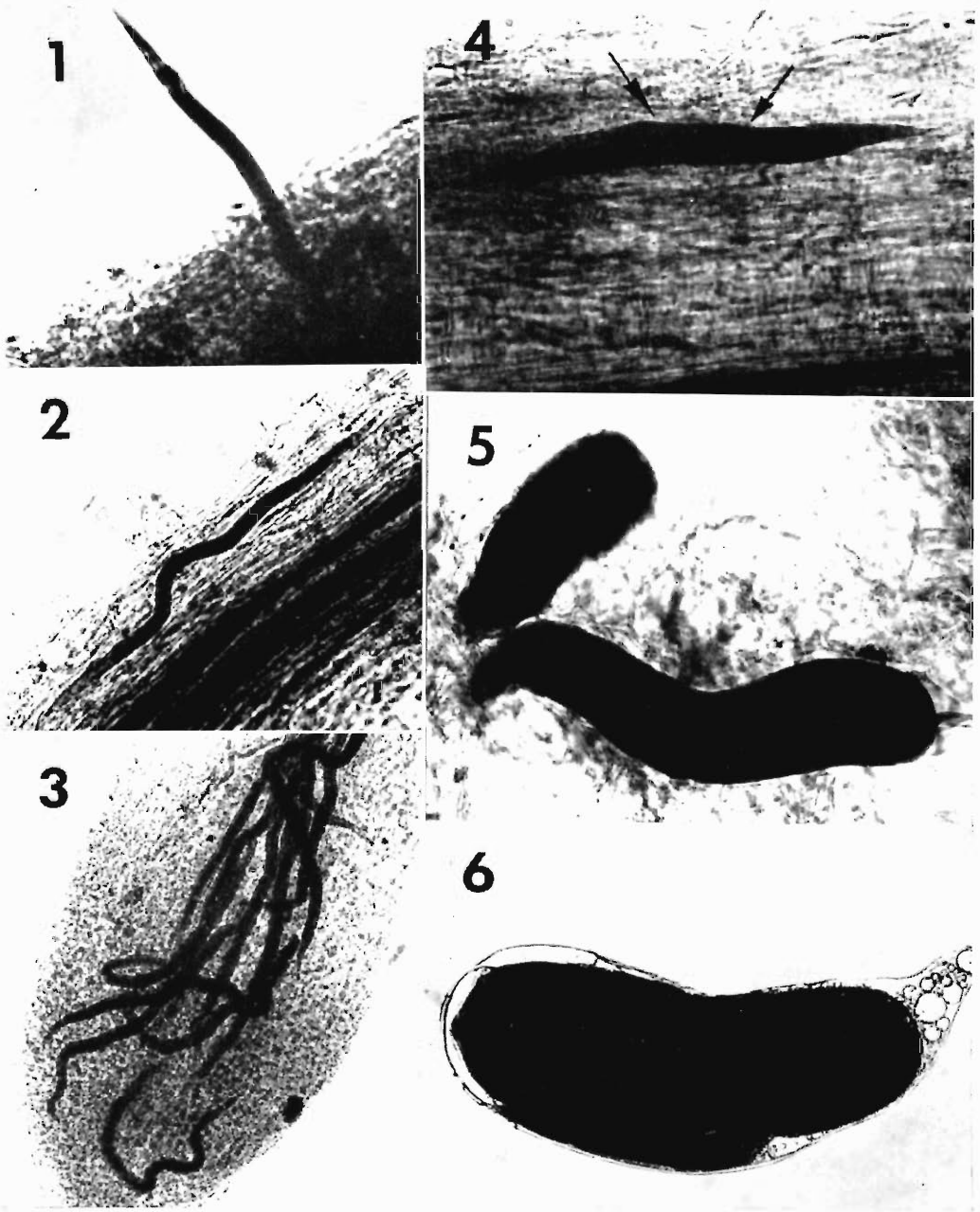
The nematode inoculum used in this study was originally obtained from soybean roots infected with *M. javanica.* A single egg mass of an identified female was isolated and the hatched larvae were then reared on tomato plants to obtain the required inoculum.

Soybean seeds of the variety N. C. Hampton were planted in steamed sandy loam soil in 24 clay pots, 15 cm diam. After emergence, the seedlings were thinned to two per pot, and then inoculated with 1,000 second-stage larvae 7 days after sowing. Pots were kept outdoors (day temperature 30 ± 2 C, night temperature 18 ± 2 C) and watered every other day. Seedlings of two pots were pulled gently at 1, 2, 4, 6, 8, and 10 days after inoculation and then at 5-day intervals up to the end of the experiment which proceeded for 40 days.

Galled parts of the infected roots were killed and fixed in FAA. For study of the nematode life cycle, parts of the fixed roots were stained with a modified Flemming's formula recommended by Oteifa and El Gindi (1956). The stained materials were washed in running water for 3 hr, then dehydrated in an aqueous ethyl alcohol series. Finally, the dehydrated root materials were cleared in clove oil and mounted in Canada balsam.

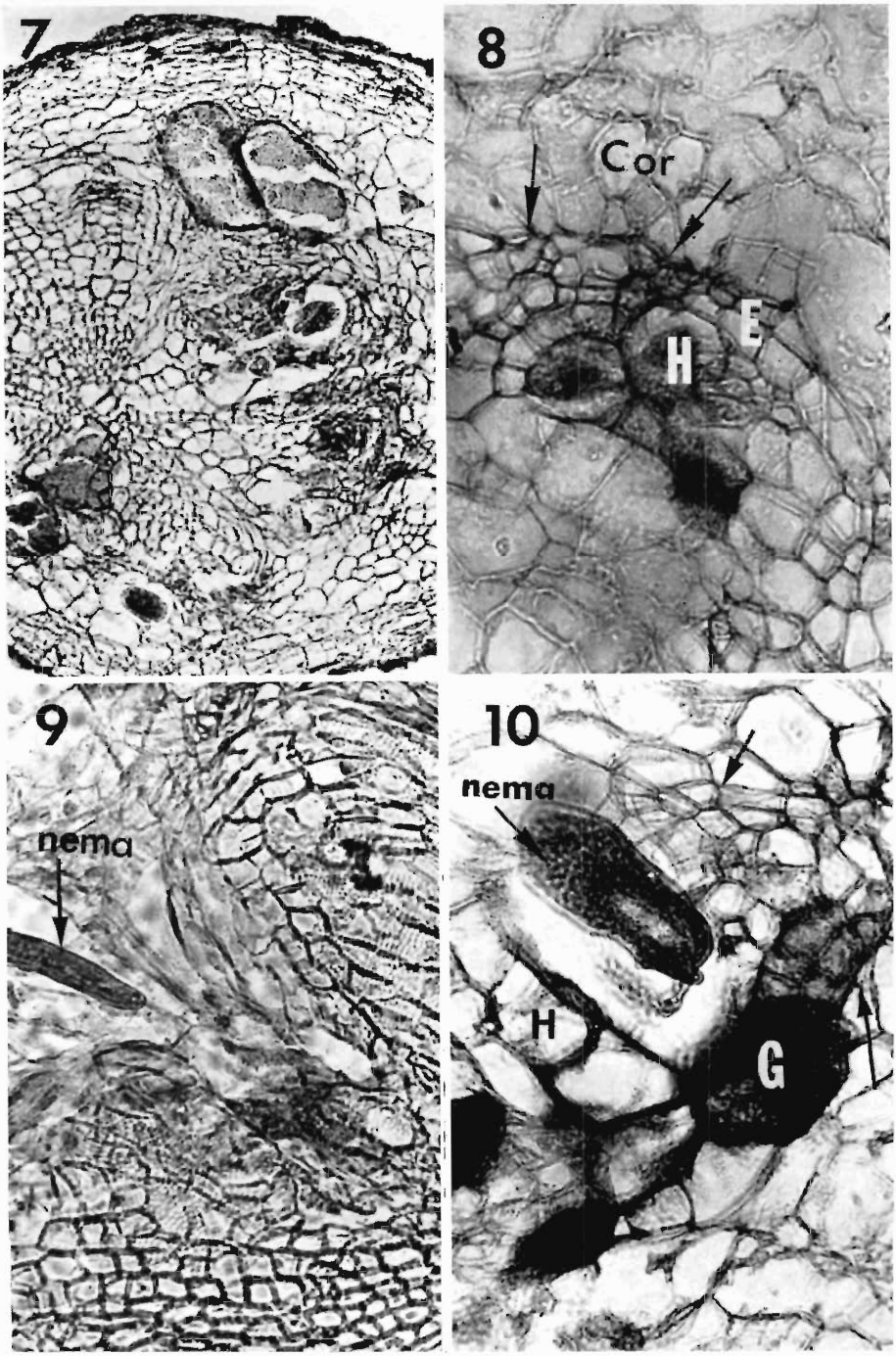
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Figures 1–6. Penetration and development of *Meloidogyne javanica* larvae in soybean roots. 1. Infective second-stage larva penetrating the root tip. 2. Second-stage larva migrating in the cortex. 3. Group of second-stage larvae in the root tip. 4. Third-stage larva in the cortex (arrow). 5. Fourth-stage larvae feeding on the vascular tissue. 6. Isolated fourth-stage male larva.

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In a histopathological study, the fixed infected roots were dehydrated in an ethyl and butyl alcohol series after which they were embedded in paraffin wax, sectioned, and stained with safranin and light green.

All sections were examined under a light microscope.

Results

Microscopic observations of stained soybean roots revealed the gross morphology of infected root tissues and the different developmental stages of *M. javanica* (Figs. 1–10). One day after inoculation, infective second-stage larvae were found penetrating the roots at or near the root tips (Fig. 1). Larval passage through the root tissues was intercellular and intracellular. Direct larval penetration of the epidermis and cortex distorted and killed nearby cells. At 2 days, second-stage larvae were observed feeding on meristematic and cortical tissues and as many as 30 larvae were seen feeding on a single root tip (Figs. 2, 3). Larvae were oriented in various directions, but more were arranged parallel to the longitudinal axis of the root with their posterior ends extended toward the root tip (Fig. 2). Within 2 days, hypertrophy of cortical parenchyma and endodermal cells occurred about the larval head. In 4 days, cells immediately surrounding the hypertrophied cells and nematode mouth were stimulated to divide and form hyperplastic tissue (Fig. 8). In 6 days, third-stage larvae were observed mostly in the inner cortex feeding on tissues of the endodermis, pericycle, and outer stele. Hypertrophied and hyperplastic cells surrounding the larval head marked the beginning of giant cell formation. Some of the affected cells contained granular materials and their cell walls were partially thickened and darkly stained (Fig. 9). In 8-day infections, walls between enlarged divided cells started to break down. Within 8 to 10 days, the majority of larvae had reached the third stage of development (Fig. 4). In 15 days, fourth-stage larvae were observed feeding on the pericyclic and stelar tissues, especially protoxylem poles (Figs. 5, 10). As many as seven giant cells were found around the head of the fourth-stage larvae. Abnormal xylem vessels, crushed and necrotic cells adjacent to developing females, and giant cells were seen in the roots. In 20 days, cell wall dissolution of adjoined giant cells was seen and resulted in the formation of openings between adjacent giant cells.

The fourth-stage male larva appeared typically as an elongated thread whereas the preadult female stage had an elongated, round body (Figs. 5, 6). In 20 and 25 days following inoculations, respectively, young and mature males and females were observed in infected root tissues. Nematode females oviposited 30 days after inoculation. Eggs of *M. javanica* collected from egg masses were observed to hatch in water at room temperature after 4–6 days. Second-stage larvae of the second generation appeared in the roots 35 days after inoculation.

Discussion

The results showed that roots of soybean seedlings became infected with second-stage larvae of *M. javanica* within 24 hr after inoculation. The seedling root tips were the most favorable parts for nematode infection. Similar results were found by Christie (1936), who reported that invasion by root-knot nematode larvae was limited mainly to root tip regions with comparatively undifferentiated tissues. The results also indicated that progressive development of the nematode larvae and presence of adults were associated with anatomical changes of the root tissues, especially in the formation of giant cells. The observed cellular disorganization of infected roots could be related to migration, feeding, and development of the infecting nematode. The histopathological changes described in this paper were

←

Figures 7–10. Cross sections of soybean roots infected with *M. javanica*. 7. Heavily infected root, 35 days after inoculation. 8. Cellular hypertrophy (H) and hyperplasia (arrow) in the cortex (Cor), endodermis (E), and stelar tissue. 9. Third-stage larva feeding on the pericycle and the vascular tissue. Note cellular disorganization around the nematode (nema) head. 10. Infected root, 15 days following inoculation, showing the formation of hypertrophy (H), hyperplasia (arrow), and giant cells (G) around the nematode feeding site. Note dark-stained granular materials inside the giant cell.

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similar to those reported previously (Christie, 1936; Davis and Jenkins, 1960; Dropkin and Nelson, 1960; Sasser and Taylor, 1952).

It is evident that nematode development and the subsequent degeneration of root tissues often resulted in the formation of large areas of necrotic cells and vacant areas, especially in the cortex. These vacant areas were not invaded by a growth of rejuvenated parenchyma cells.

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Reciprocal Transfer of *Heterakis gallinarum* Larvae between Ring-necked Pheasants and Japanese Quail: Effects on *H. gallinarum, Histomonas meleagridis,* and *Parahistomonas wenrichi*

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ABSTRACT: The results of the reciprocal transfer of 10-day *Heterakis gallinarum* larvae between the ring-necked pheasant (a very compatible host) and the Japanese quail (a poor host) were compared with the results following the transfer of 10-day larvae to homologous hosts in each instance and with the results for undisturbed worms in each host species. Worms that spent their first 10 days in pheasants continued to thrive in pheasants, but failed to persist in quail. The worms that matured in the recipient pheasants were significantly larger than those that matured undisturbed in pheasants. Recovery of 10-day heterakid larvae from Japanese quail was exceedingly poor, but of the 36 that were transferred to pheasants, 20 developed to maturity. As in the pheasant-to-pheasant transfers, surviving worms were larger than those that developed in pheasants without being disturbed and contained more eggs that embryonated. Apparently, the adverse effects of spending the first 10 days in the Japanese quail, an unfavorable host, had not prejudiced the subsequent development of the survivors that were placed in the pheasant, a favorable host. However, the good start afforded larvae by living 10 days in the pheasant did not enhance their chances of survival and development in the quail.

In various previous studies, we have transferred young *Heterakis gallinarum* from birds of one species to those of the same or another species, noting the effects of such transfer on the retention and development of the heterakids (Lund, 1972; Lund and Chute, 1972b, 1973a; Lund and Chute, 1973b). The two host species never differed sufficiently in their innate compatibility to *H. gallinarum* to produce conspicuous differences between the results of transfer to birds of the same species (homologous transfers) and those of transfers to birds of the other species (heterologous transfers). Some strains of *H. gallinarum* find chickens, turkeys, and even chukar partridges to be almost equally satisfactory as hosts. However, we have never encountered a strain of *H. gallinarum* that did poorly in ring-necked pheasants (*Phasianus colchicus*), nor have we known one to do well in Japanese quail (*Coturnix coturnix japonica*). Therefore, these two species of galliform birds appeared to be satisfactory for determining the heterakid's ability to adapt itself to the change from a favorable to an unfavorable environment or the reverse.

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Specifically, we were interested in determining: (1) whether the effects of early development in the relatively incompatible host (the Japanese quail) would be permanently impressed on the heterakids, and (2) if their tissue stage was spent in the compatible host (the ring-necked pheasant), whether the adverse influences of the relatively incompatible host would be as detrimental to worms that were to encounter them only as lumen dwellers in the ceca. We also investigated the effects of transfer on the liberation of *Histomonas meleagridis* and *Parahistomonas wenrichi* and the transmission of *H. meleagridis.*

Materials and Methods

To avoid the possible influence of physiological adaptation to a particular host (Lund et al., 1970), eggs of *Heterakis gallinarum* from naturally infected turkeys, chickens, and pheasants were pooled. The worms were then grown in New Hampshire chickens for one generation, during which we determined that they transmitted both *Histomonas meleagridis* and, more frequently, *P. wenrichi.* The inoculum was prepared as previously described (Lund and Chute, 1972b) from eggs in the heterakids recovered from the chickens.

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Table 1. Results of reciprocal transfer of 10-day-old *Heterakis gallinarum* larvae between ring-necked pheasants and Japanese quail. Each control and donor bird was given 220 embryonated heterakid eggs.

	Pheasant controls	Donors			Quail controls	
		Pheasants		Quail		
No. of birds	10	20		20	10	
Recovery of 10-day *Heterakis*, %	—	82		4	—	
Incidence of *Histomonas* at 10 days, %	—	25		15	—	
		Recipients				
		Pheasants	Quail	Pheasants	Quail	
No. of birds	(9*)	10	10	4	2	(10)
Avg no. of larvae transferred	—	161	174	9	52	—
Recovery of 38-day *Heterakis*, % of:						
220 eggs	40	33	0	2	0	0.1
Larvae transferred	—	46	0	56	0	—
Avg length of 38-day *Heterakis*, mm:						
Females	10.9	11.7	—	11.8	—	9.0
Males	8.8	9.5	—	9.5	—	6.5
Avg no. embryonated eggs per female	47	48	—	99	—	22
Incidence by 38 days, %						
Histomonas	33	30	0	0	0	0
Parahistomonas	89	80	10	0	0	0
Embryonated *Heterakis* eggs fed per *Histomonas* infection in test poults:						
Fed as pooled eggs	470	118	—	—	—	—
Fed in intact females	237	158	—	135	—	—
Average	354	138	—	—	—	—

* One pheasant control died early of causes unrelated to the experimental procedure.

Both the ring-necked pheasants and the Japanese quail were from flocks reared at the Animal Parasitology Institute for several years. All birds were raised and maintained free of extraneous infections with heterakids and histomonads and were 6 to 7 weeks old as the tests started. They were caged individually to permit the certain identification of their cecal discharges for microscopic examination.

Each of 30 pheasants and 30 Japanese quail was given approximately 220 embryonated heterakid eggs in 0.85% saline by pipette to the crop. Ten of the pheasants and 10 of the Japanese quail were designated as controls, in which the heterakids were left to develop undisturbed. The other 20 birds of each species were killed as donors of 10-day heterakid larvae to be transferred to recipient hosts by rectal inoculation as described previously (Lund and Chute, 1972b). In each instance, 10 donors provided larvae for recipients of the same species (i.e., pheasants to pheasants or Japanese quail to Japanese quail) and the remaining 10 donors provided larvae for recipients of the other species (pheasants to Japanese quail, Japanese quail to pheasants).

The cecal discharges of each bird were examined for histomonads as described previously (Lund and Chute, 1973a). Control and recipient birds were necropsied when their heterakids were 38 days old (for recipients, this was 28 days after receiving 10-day larvae). The worms were removed from each bird, counted, sexed, and measured; females were kept in 0.5% formalin at room temperature to permit embryonation of eggs. The average number of embryonated eggs per female, and the ability of such eggs to transmit *H. meleagridis* to turkeys, were determined as described previously (Lund and Chute, 1972c).

Results

The results are summarized in Table 1. Recovery of 10-day heterakids was high in the pheasant donors and very low in the Japanese quail donors. Because only six of the quail had any larvae, and because each donor had arbitrarily been assigned to a given recipient host, only four pheasants and two Japanese quail received 10-day larvae from Japanese quail donors.

The recoveries of mature heterakids from recipients of pheasant-to-pheasant and quail-to-quail transfers, based on the number of embryonated eggs given to the donors, were similar to the recoveries of undisturbed worms

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from controls of the appropriate species. Recovery calculated as a percentage of the larvae actually transferred was high in both groups of recipient pheasants, but no worms survived to maturity in either group of recipient Japanese quail. Both male and female heterakids in pheasant-to-pheasant recipients were significantly (Student's t-test, $P < 0.01$) larger than those in the control pheasants. Even those worms that had been transferred from Japanese quail to pheasants were larger than the undisturbed worms in the control pheasants.

The increase in incidence of infection with *Histomonas meleagridis* that has occurred in recipient chickens and turkeys, compared with controls (Lund and Chute, 1972b), did not occur in either species on this test. *Parahistomonas wenrichi* was found almost exclusively in the control pheasants and the recipients of pheasant-to-pheasant transfers. This protozoan has never been reported from the Japanese quail but was found in one of the quail that received 10-day larvae from a pheasant donor.

As in previous studies (Lund and Chute, 1972b, 1973a; Lund and Chute, 1973b) fewer eggs from transferred worms than from undisturbed worms were required to produce an infection with *H. meleagridis* in young poults.

Discussion

Although the ring-necked pheasant is the most compatible host for *Heterakis gallinarum* that we have studied (Lund and Chute, 1972a, c, d), there can still be a very substantial loss of worms between the 10th and 38th days. In the control pheasants, this loss averaged 42% (82% remaining at 10 days; 40% at maturity) of the 220 embryonated eggs given, more than twice the 18% loss during the first 10 days. The latter figure is remarkably low, considering that hatching, transport into the cecum, entry (total or partial) into the mucosa, a molt, emergence into the lumen, migration towards the distal end of the cecum, and another molt all are required during the first 10 days.

The hazards of the next 28 days include additional migration to the distal end of the cecum, another molt (usually at 14 days), finding enough food to meet the requirements of a 15- to 25-fold increase in mass and activity sufficient to resist the twice-a-day evacuation of the cecum, and mating. To these must be added the effects of any immunity developed by the host in response to invasion of its cecal tissues by the heterakid larvae. Whatever their nature, these immune responses in pheasants are certainly operative by the 21st day of infection with *H. gallinarum* (Lund and Chute, 1972a) and are at least the equal of those in chickens and turkeys (Lund, 1967). Their effects may still be negligible by the 10th day but probably become important before the 21st day. In most individuals of some species of galliform birds, the young worms are clearly in difficulty by the 14th day and all but gone by the 17th day (Lund and Chute, 1971a, b, 1972c).

Birds receiving 10-day larvae are never exposed to the stimulus of tissue invasion. Thus, any immune responses in recipient birds are because of the presence of the worms in the cecal lumen and should be much weaker than those in birds which have harbored *H. gallinarum* in their cecal mucosa. However, the hazards of transfer are great (Lund, 1972; Lund and Chute, 1972b), even exceeding the hardships imposed on heterakids developing to maturity in a single bird. The selective influences operating on transferred worms may in no way enhance the survivors' ability to perpetuate themselves indefinitely in galliform birds but, presumably, do eliminate the less hardy individuals, so at maturity transferred worms of both sexes are larger than their undisturbed counterparts.

The Japanese quail is a very poor host for both *Heterakis gallinarum* and *Histomonas meleagridis* (Lund and Ellis, 1967; Lund and Chute, 1972c). The extremely low recovery of heterakids at 10 days, together with the low incidence of infection with *H. meleagridis*, could mean that neither parasite finds the Japanese quail nutritionally satisfactory or that the quail has a high natural resistance to both parasites. Hatching and retention of *Heterakis gallinarum* may usually be so poor that histomonads are liberated too infrequently to result in detectable infections.

Apparently, the adverse effects of the early environment in the Japanese quail were not permanently impressed on those larvae that survived to be transferred to the more compatible host, the pheasant. Twenty (56%) of the 36 larvae so transferred survived to 38 days, a slightly higher percentage than survived the pheasant-to-pheasant transfer and

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also better than hitherto recorded following transfer into a different species of host (Lund, 1972; Lund and Chute, 1972b, 1973a). Although only eight of these surviving worms were females, they were large, and averaged twice as many eggs capable of embryonation as did the females in control pheasants and recipients of pheasant-to-pheasant transfers. We consider the stringent selection to have accounted for this; only the most hardy worms survived the ordeal of living 10 days in the Japanese quail and that of transfer.

Neither of the two Japanese quail given 10-day larvae from Japanese quail had any heterakids 28 days after the transfer. However, 14 of the 20 prospective donor quail had none at 10 days, and nine of the 10 control quail had none at 38 days. Since 77% of the quail failed to yield heterakids when no transfer had been involved, we cannot draw any conclusions concerning the effects of quail-to-quail transfers.

Starting the heterakids in a compatible host, the pheasant, afforded them no advantage following transfer into the relatively incompatible Japanese quail.

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Eimeria tenella: An in vivo and in vitro Comparison of the Wisconsin, Weybridge, and Beltsville Strains

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ABSTRACT: The Wisconsin, Weybridge, and Beltsville strains of *Eimeria tenella* were compared as to their pathogenicity and oocyst production in vivo and infectivity and oocyst production in vitro. In chickens, the Wisconsin strain caused the greatest reduction in weight gain, but the Weybridge strain caused the highest mortality. Cecal lesion scores were similar. Most oocysts were produced by the Wisconsin strain and fewest by the Beltsville strain. In primary chicken kidney cell cultures, the percentage infection with the Wisconsin strain 4 hr after inoculation was higher than with the other two strains, but oocyst production at 6, 7, and 8 days was lower. The Beltsville strain produced the most oocysts in cell culture.

Recent studies (Joyner and Norton, 1969; Long, 1970) have shown that the Weybridge and Houghton strains of *Eimeria tenella* differ in pathogenicity. In the chicken, the Weybridge strain caused greater cecal hemorrhage than did the Houghton strain when sublethal dosages of 625 oocysts were given (Joyner and Norton, 1969); in the embryonated chicken egg, with inocula containing 5, 15, and 45 thousand excysted sporozoites, the Weybridge strain produced higher percentages of mortality (Long, 1970).

This paper compares the Weybridge, Beltsville, and Wisconsin strains of *E. tenella* as to (a) pathogenicity and oocyst production in chickens and (b) infectivity and oocyst production in primary chicken kidney cell cultures.

Materials and Methods

Oocysts

The Weybridge strain was obtained from Dr. R. F. Shumard, Eli Lilly and Company, Greenfield, Indiana, who had previously obtained the strain from Dr. L. P. Joyner, Central Veterinary Laboratory, Weybridge, England. The Wisconsin strain was obtained from Dr. T. K. Jeffers, Hess and Clark, Ashland, Ohio. The Beltsville strain was isolated from a field outbreak at the Agricultural Research Center, Beltsville, Maryland, in 1938 by M. M. Farr, and has been maintained by laboratory passage since that time.

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In order to eliminate as many variables related to oocyst age as possible, 25 chicks per strain were inoculated with 1×10^3 oocysts. Eight days later, the ceca were removed, homogenized in a Waring blender with 2.5% $K_2Cr_2O_7$, and the oocysts allowed to sporulate. One week later, 100 chicks per strain were given 1.2×10^3 of the newly sporulated oocysts by gavage. The oocysts produced were collected, sporulated, and cleaned of fecal debris as previously reported (Vetterling, 1969). After treatment with 5.25% sodium hypochlorite (undiluted Clorox) for 20 to 30 min, oocysts of each strain were washed 3 to 5 times with sterile distilled water, resuspended in 250 ml of Ringer's solution, counted, and stored at 3 to 6 C. Oocysts in these stock cultures were 6, 11, and 15 weks old when used in experiments 1, 2, and 3, respectively.

Inoculation and treatment of chicks

One-day-old California White cockerel chicks were obtained from a hatchery and raised on wire mesh in electrically heated brooders in an isolation building. They were fed on all-mash, unmedicated ration (ARC Broiler Diet A). Droppings were examined weekly for oocysts. When 3 weeks old, the chicks were weighed, randomly divided into cage groups of six, placed in Hartford poultry batteries, and given oocysts by gavage. Five cage groups (30 chicks) were used for each strain and uninoculated controls.

Approximately 9 million oocysts of each strain were removed from stock cultures and

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Table 1. Mortality, weight gain, cecal lesions, and oocyst production in chicks infected with different strains of *Eimeria tenella*.

Strain	Exp. No.	No. of birds Start/End	% Mortality	Bird weight (g) Start* x̄ ± SE		End x̄ ± SE		Weight gain (avg)	Lesion score (avg)	Oocysts/bird × 10³ x̄ ± SE	
Uninoculated	1	30/30	0	206.2	1.07	336.8	1.25	130.6	—	—	
	2	30/30	0	206.3	1.06	334.5	1.25	128.2	—	—	
	3	30/30	0	208.8	1.05	332.2	1.23	123.4	—	—	
		90/90	0	207.1	1.06	334.5	1.24	127.4	—	—	
Wisconsin	1	30/21	30	208.8	0.99	210.3	1.41	1.5	2.8	5.7	0.055
	2	30/14	53	207.3	0.99	233.6	1.72	26.3	2.9	2.5	0.036
	3	30/22	27	209.2	1.03	248.2	1.41	39.0	2.8	2.6	0.037
		90/57	37	208.4	1.00	230.7	1.51	22.3	2.8	3.6	0.044
Weybridge	1	30/16	47	209.0	1.08	250.0	1.73	41.0	3.0	3.7	0.045
	2	30/7	77	208.2	1.15	257.3	2.75	49.1	3.1	2.7	0.038
	3	30/14	53	205.0	1.02	250.1	1.77	45.1	3.2	2.9	0.039
		90/37	59	207.4	1.08	252.2	2.08	45.1	3.1	3.1	0.041
Beltsville	1	30/20	33	205.1	1.07	258.0	1.54	52.9	2.9	2.5	0.037
	2	30/23	23	206.5	1.02	252.3	1.38	45.8	2.8	1.8	0.031
	3	30/22	27	208.3	1.08	252.8	1.48	44.5	2.9	1.4	0.027
		90/65	28	206.6	1.06	254.4	1.46	47.7	2.9	1.9	0.032

* All starting weights statistically similar ($P < 0.05$).

diluted with Ringer's solution until 1 ml was thought to contain approximately 1×10^5 oocysts. The numbers of oocysts in these suspensions were then determined by the method of Long and Rowell (1958) and the suspensions were adjusted until the counts were statistically the same ($P < 0.05$). One ml of oocyst suspension of each strain was given to each of 30 birds in each of three experiments. Oocyst suspensions contained the following numbers of oocysts: Weybridge strain, $108 \pm 4.7 \times 10^3$; Beltsville strain, $104 \pm 3.2 \times 10^3$; Wisconsin strain, $106 \pm 4.3 \times 10^3$.

Chicks were weighed and killed 8 days after inoculation. Ceca of infected chicks were removed. After lesions were evaluated by an objective four-point scoring system (McLoughlin and Chute, 1966), the ceca were homogenized in a Waring blender. Oocysts in each homogenate were counted in a McMaster's paracytometer using the method described by Long and Rowell (1958) in which two chambers for each sample and 10 samples for each cage-homogenate were counted.

Mortality of infected chicks was compared by Chi-square analysis. The mean weights, cecal lesion scores, and oocyst counts were evaluated statistically by Student's t test either one-sided or two-sided as applicable (Simpson et al., 1960). In all cases, a probability of 0.05 or less was considered significant.

Inoculation and treatment of cell cultures

Kidneys were obtained from 3-week-old chicks. The procedure for trypsinizing minced kidney tissue, treating cells after trypsinization, and establishing cultures were the same as previously described (Doran, 1971). Cells were grown in a medium consisting of 80% Hank's balanced salt solution (HBSS), 10% lactalbumin hydrolysate (LAH, 2.5% solution in HBSS), and 10% fetal calf serum. In each experiment, 20 Leighton tube cultures (10.5- by 35-mm cover slip) were prepared for inoculation with each strain of *E. tenella*.

Two days after cell cultures were established, and on the same day that birds were inoculated, 10 times more oocysts than the theoretical number needed to inoculate the 20 tubes with 1×10^5 sporozoites were removed from the stock cultures. Sporocysts were released from these oocysts by grinding (Doran and Vetterling, 1968), washed twice with Ringer's solution, and treated with trypsin–bile solution at 43 C for 1 hr. Excysted sporozoites were separated from debris (intact oocysts and sporocysts; oocyst and sporocyst walls) by passage through columns (240 by 15 mm) of packed glass beads (100 μm diam). Sporozoites of each strain were then resuspended in 50 ml of a medium containing 90% HBSS, 5% LAH, and 5% fetal calf serum and counted with a counting chamber. Suspensions with

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Table 2. Percentage infection and oocyst production in primary chicken kidney cell cultures infected with different strains of *Eimeria tenella*.

Strain	Exp. No.	Sporozoites (4 hr) Sporozoites counted	% infection	Oocysts (6 days) Number counted	OI	Oocysts (7 days) Number counted	OI	Oocysts (8 days) Number counted	OI	No. in media/ tube
Wisconsin	1	266 (15) *	46	14 (45)	0.053	21 (56)	0.079	22 (48)	0.083	0
	2	194 (17)	40	1 (65)	0.005	6 (50)	0.030	5 (45)	0.025	0
	3	163 (15)	22	4 (14)	0.006	12 (35)	0.074	7 (100)	0.043	0
			36		0.021		0.061		0.051	
Weybridge	1	140 (21)	31	16 (55)	0.174	78 (44)	0.557	61 (89)	0.436	675
	2	82 (23)	15	1 (45)	0.012	12 (45)	0.145	14 (67)	0.171	1,000
	3	275 (9)	12	1 (55)	0.004	7 (45)	0.025	11 (67)	0.040	333
			19		0.063		0.242		0.261	
Beltsville	1	197 (10)	31	74 (34)	0.375	183 (38)	0.929	221 (55)	1.629	1,750
	2	97 (14)	20	6 (39)	0.062	25 (55)	0.257	24 (45)	0.247	1,000
	3	137 (16)	18	3 (25)	0.021	54 (63)	0.393	57 (50)	0.416	666
			23		0.153		0.526		0.764	

* Numbers in parentheses represent per cent deviation (avg.) between three to five cover slips.

the higher counts were diluted so that the numbers of sporozoites per ml were within 10% of the number per ml in the suspension with the lowest number of sporozoites. The 1-ml inoculum for each of the 20 culture tubes used per strain contained $101 \pm 6.2 \times 10^3$ sporozoites in experiment 1, $25 \pm 4.0 \times 10^3$ in experiment 2, and $46 \pm 3.9 \times 10^3$ in experiment 3.

An additional 9 ml of the same medium used for inoculation was added to each tube 3 hr after inoculation. Cultures were kept at 41.5 C. At 4 hr and at 6, 7, and 8 days, cover slips were removed from three to five cultures inoculated with each strain. Cells were fixed in 10% neutral buffered formalin and stained as previously described (Doran and Vetterling, 1967). Counts were made of the number of sporozoites at 4 hr and oocysts at 6, 7, and 8 days in 240 microscopic fields. At 4 hr, the percentage of each field covered with cells was estimated and the percentage confluency of cells was calculated. The location of the fields examined was the same as before (Doran, 1971). Percentage infection was calculated using the same equation as before (Doran, 1971) except that PC (percentage confluency) was divided by 100. The oocyst index (OI) was calculated by dividing the number of oocysts counted by the number of sporozoites counted at 4 hr. At 8 days, the medium in tubes from which cover slips had been removed was pooled and centrifuged at 250 *g* for 10 min. After decanting to 2 ml, counts were made and the number of extracellular oocysts per culture was determined.

Results

In chicks, strains differed in their effect on mortality and weight gain and in the quantity of oocysts produced (Table 1). The Weybridge strain caused greater mortality than either the Beltsville or Wisconsin strains ($P < 0.01$). On the other hand, the Wisconsin strain caused much greater reduction in weight gains than the other two strains ($P < 0.005$). Cecal lesion scores were similar ($P < 0.60$). Oocyst production varied considerably between experiments, but, when averaged, most oocysts were produced by the Wisconsin strain and the fewest by the Beltsville strain ($P < 0.01$).

Strains also differed in cell culture (Table 2). Percentage infection with the Wisconsin strain was higher than with the other two strains. However, oocyst production with the Wisconsin strain was always lower. With this strain, few oocysts were found in the cells and none in the medium. The Beltsville strain, which produced the smallest number of oocysts in the chicken, produced the most in cell culture.

Discussion

If weight gain is used as the sole criterion of pathogenicity, the Wisconsin strain was the most pathogenic; if mortality is used, then the Weybridge strain was most pathogenic.

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Based on both criteria, the Beltsville strain was the least pathogenic. When the Wisconsin strain was sent to us, it was thought to be the "least pathogenic" strain available (T. K. Jeffers, pers. comm.). The difference in the degree to which this strain affected chicks in the present study might be due to vitamins in the feed. Warren (1968) showed that dietary vitamins have an effect on the degree of pathogenicity of *E. tenella*. The significant difference between strains in oocyst production was also unexpected. Joyner and Norton (1969) found oocyst production by the Weybridge and Houghton strains to be essentially similar.

The greater percentage infection with the Wisconsin strain in cell culture suggests that this strain may have a greater capacity for entering cells. Even though more sporozoites of this strain were found intracellular at 4 hr, only a few oocysts were found later. There may be a direct relationship between pathogenicity of a strain and magnitude of oocyst production in cell culture. In addition to there being fewer Wisconsin strain oocysts, at 6 days in all three experiments there was also a noticeably smaller number of macrogametes, and, especially, second-generation schizonts. Perhaps some time before 6 days schizonts of the Wisconsin strain destroyed more of the monolayer host cells than other strains before the potential gametocyte-producing merozoites within could mature.

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Intestinal Helminths of the White Sucker, *Catostomus commersoni* (Lacépède), in SE Wisconsin

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ABSTRACT: Five species of helminths were recovered from the intestine of the white sucker, *Catostomus commersoni* (Lacépède), in southeastern Wisconsin. Hosts were seined in five sites in both the Root River (Milwaukee and Racine counties; autumn 1971) and the Pike River (Racine and Kenosha counties; autumn 1972). The helminths are *Triganodistomum attenuatum* Mueller and Van Cleave, 1932 (Trematoda: Lissorchiidae), new locality record; *Biacetabulum macrocephalum* McCrae, 1962 (Cestoda: Caryophyllaeidae), new state record; *Biacetabulum biloculoides* Mackiewicz and McCrae, 1965 (Cestoda: Caryophyllaeidae), new state record; *Acanthocephalus dirus* (Van Cleave, 1931) Van Cleave and Townsend, 1936, new host and state record; *Dorylaimus* sp. (?) Dujardin, 1845 (Nematoda: Dorylaimidae), a nonparasitic nematode reported for the first time in this fish. Distribution, structural observations, and host–parasite relationships of the above species are discussed.

Previous reports of fish parasites in Wisconsin were confined to the geographical northeast and west (Pearse, 1924a), north (Bangham, 1946), northwest (Fischthal, 1947, 1950, 1952), and east (Anthony, 1963). These reports dealt only with host records. Literature on the ecology or host–parasite relationship of fish parasites in Wisconsin is relatively scarce (Marshall and Gilbert, 1905; Pearse, 1924b; Cross, 1938).

Current efforts are devoted to the examination of various fish hosts, in the previously uninvestigated southeastern portion of Wisconsin. These surveys also include structural and ecologic observations and host–parasite relationships. *Lernaea cyprinacea* Linn. (Copepoda: Crustacea) has been recently reported from 10 fish species including *C. commersoni* in southeastern Wisconsin by Amin et al. (1973). The present report deals with five helminths recovered from the intestine of the white sucker.

Materials and Methods

A total of 321 suckers was seined from five sites each in the Root River (Racine and Milwaukee counties, autumn 1971, 186 fish) and the Pike River (Racine and Kenosha counties, autumn 1972, 135 fish). Both rivers drain into Lake Michigan. Fish were initially placed in 10% formalin, later transferred to 70% ethyl alcohol, and then examined for parasites which upon recovery were placed in 70% alcohol. Hosts were classed in one of three size classes according to their total length, 5–9.9, 10–14.9, and 15–37 cm.

Trematodes and cestodes were stained in Semichon's carmine, cleared in xylene, and whole-mounted in Canada balsam. Acanthocephalans were fixed in Bouin's fluid, stained in Harris' hematoxylin, cleared in beechwood creosote, and whole-mounted in Canada balsam. Nematodes were not permanently mounted. They were cleared in glycerol which was added to the nematode containing vials in equal amounts to the alcohol present. A period of 2 to 4 weeks and warm temperatures were necessary for the complete evaporation of alcohol and the penetration of glycerol.

Whole-mounted trematodes, cestodes, and acanthocephalans were initially nonrandomly selected for mounting and measuring, based on their representing the variable sizes and shapes of recovered specimens.

Results and Discussion

Throughout the text the term "young adults" is used for small adults with incompletely developed gonads. The term "mature adult" applies to older (gravid) worms with fully developed reproductive systems. All measurements are in millimeters unless otherwise specified. Mean figures represent the number of worms recovered/number of hosts examined.

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Table 1. Parasite load and host size relationships of five helminths recovered from the intestine of the white sucker in the Root River, autumn 1971, and Pike River, autumn 1972, southeastern Wisconsin.

Stream	Parasitic species	Host size (total length) (cm)	No. hosts examined	% infect.	No. of parasites recovered	Mean/host	Max. no./host	% mature adults
Root River	*Triganodistomum attenuatum*	5– 9.9	160	36	175	1.1	38	26
		10–15	17	18	5	0.3	2	100
		20–37	9	—	0	—	0	—
		Total	186	33	180	1.0	38	28
	Biacetabulum macrocephalum	5– 9.9	160	19	113	0.7	55	2
		10–15	17	12	4	0.2	2	75
		20–37	9	55	7	0.8	2	14
		Total	186	20	124	0.7	55	5
Pike River	*Triganodistomum attenuatum*	5– 9.9	58	12	10	0.2	2	90
		10–14.9	72	3	2	0.03	1	100
		15–31	5	20	1	0.2	1	100
		Total	135	7	13	0.1	2	92
	Biacetabulum biloculoides	5– 9.9	58	12	16	0.3	4	88
		10–14.9	72	28	34	0.5	4	94
		15–31	5	20	1	0.2	1	100
		Total	135	20	51	0.4	4	92
	Acanthocephalus dirus	5– 9.9	58	14	32	0.5	17	100
		10–14.9	72	37	48	0.7	7	100
		15–31	5	100	66	13.2	20	100
		Total	135	29	146	1.1	20	100
	Dorylaimus sp. (?)	5– 9.9	58	16	82	1.4	40	100
		10–14.9	72	36	1,010	14.0	150	100
		15–31	5	—	0	—	—	100
		Total	135	26	1,092	8.0	150	100

Triganodistomum attenuatum Mueller and Van Cleave, 1932

Distribution

This trematode has been reported from British Columbia, Iowa, Michigan, New York, Ohio, Wisconsin, and Wyoming by various authors as noted in Hoffman (1967). In Wisconsin, it was reported from the northern, northwestern, and eastern parts of the state by Bangham (1946), Fischthal (1947, 1950, 1952), and Anthony (1963). The present records, from the Root and Pike rivers of southeastern Wisconsin, represent new locality records.

Incidence in suckers from the Root River (33%) was considerably higher than from the Pike River (7%) (Table 1) but comparable to rates reported by the above authors in other white suckers elsewhere in Wisconsin.

Structural observations

A total of 193 specimens was recovered. One of the 13 Pike River specimens and 28% of the 180 Root River specimens were young adults. Whole mounts of mature adults (Fig. 1A) from *C. commersoni* taken in both rivers were 0.80–3.48 long, 0.24–0.80 in maximum width, 0.14–0.36 in oral sucker diameter, and 0.14–0.56 in ventral sucker diameter ($N = 18$). These measurements were invariably greater in worms recovered from Pike River suckers than in those from Root River suckers. Measurements of the type specimen (Mueller and Van Cleave, 1932) fell within that range, nearer to the maximum figures. Young adults (Fig. 1A) (only from Root River suckers) were 0.44–0.76 in length, 0.20–0.24 in maximum width, 0.10–0.16 in oral sucker diameter, and 0.10–0.16 in ventral sucker diameter ($N = 8$). The ventral sucker grew faster than the oral sucker to become significantly larger in older worms. My mature adults differed from those described by Mueller and Van Cleave (1932) and Mueller (1934) in having a more elongate pharynx, more transversely elongate ovary, and more variable lobation of testes (Fig. 1A). Pharynx length and width: 0.08–0.24 by 0.06–0.16. Corresponding measurements of the ovaries and testes were 0.08–0.28 by 0.08–0.36 and 0.12–0.36 by 0.14–0.32 ($N = 16$). No

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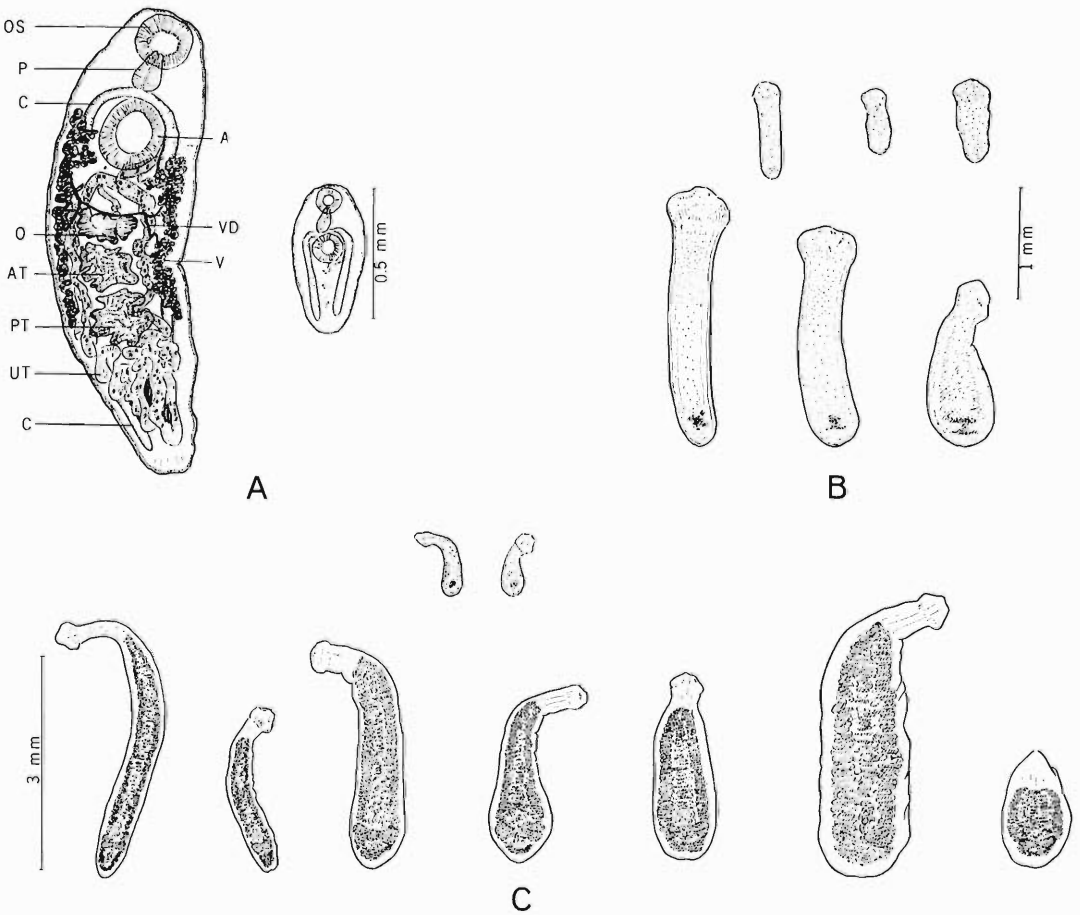


Figure 1. (A). A mature and young adult *Triganodistomum attenuatum* from a Root River white sucker (abb.: A = acetabulum, AT = anterior testis, C = cecum, O = ovary, OS = oral sucker, P = pharynx, PT = posterior testis, UT = uterus, V = vitellaria, VD = vitelline duct). (B). Structural variations in young adults presumably of *Biacetabulum macrocephalum*. (C). Structural variations in relaxed and contracted young adults (upper row, two specimens) and mature adults (lower row) of *Biacetabulum biloculoides*. The structural forms of the mature adults are drawn from left to right in order of their encountered prevalence. Drawings were made with the aid of a microprojector.

measurements or detailed descriptions of the above characters were given by Mueller and Van Cleave (1932); the variations shown by all their specimens were not indicated. The cited authors (Mueller; Mueller and Van Cleave) also did not show that the vitelline duct has two branches (an anterior and posterior branch) on either side of the body which empty into the main right and left vitelline ducts (Fig. 1A).

Effects of host on worm burden and site of infection

There was a definite relationship between the ratio of young to mature adults of *T. attenuatum*, and host size (age) and site of infection, at least in the two streams surveyed. Young adults were common in the smaller hosts (74% of total recovered from Root River suckers measuring 5–9.9 cm long) but were not recovered from larger hosts (Table 1).

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Young adults (from Root River) were recovered from the stomach (intestinal swelling, 45%), small intestine (anterior portion, 35%), and large intestine (posterior portion including rectum, 20%). Mature adults were mostly recovered from the large intestine (85%) and the remainder from the small intestine. Maturation of *T. attenuatum* appears to be correlated with progressive migration (posteriorly) in the intestinal tract of growing suckers. The site of infection with *T. attenuatum* was not mentioned by any of the above authors.

A comparison between the overall infection rates (36%, 18%, 0%) and means per host (1.1, 0.3, 0) in the three host size classes indicates a considerable decrease in worm burden by age (Table 1). The lighter infections in older hosts might indicate a possible change in host feeding habits, short-term residual infections, a host age resistance mechanism, or a combination of any of these factors.

Biacetabulum macrocephalum McCrae, 1962

Distribution

This cestode was reported only once in *Catostomus commersoni* in Iowa by Calentine (1965) since its original description from the same host in Colorado by McCrae (1962). The present report from the Root River represents a new species record in Wisconsin and extends its known range of distribution beyond the type locality.

B. macrocephalum infected 20% of the Root River suckers (Table 1) and 11% of Calentine's hosts from Iowa, but was referred to by McCrae as "quite rare" in Colorado compared to other species of caryophyllaeids.

Structural observations

A total of 124 specimens was recovered, six mature adults and 118 young adults presumably of the same species. Four whole-mounted mature adults were nearly twice as long as those originally described by McCrae and considerably longer than those by Calentine. They were 7.36–11.50 in total length, 0.72–0.92 in maximum width, 0.72–0.80 in scolex width, and 0.36–0.52 in neck width. Corresponding measurements of the McCrae adults were 4.0–6.5, 0.55–0.88, 0.63–0.83, and 0.324–0.462. The Calentine adults were 5.5–9.0 long. The same characters in my young adults were 0.44–1.96, 0.16–0.52, 0.16–0.48, and 0.12–0.36 ($N = 9$), in the same order. The range of their structural variation is shown in Fig. 1B. No young adults were reported by either McCrae or Calentine.

Site of infection

All young and mature adults were loosely attached or suspended in the stomach, with one exception. Fifteen young adults were found in the anterior portion of the small intestine in close proximity to the stomach in one host with a relatively heavy parasitic load (55 young adults). McCrae's specimens were reported from the "intestine" while Calentine's were described as firmly attached in the "gut" mucosa 2 to 6 inches posterior to the stomach.

Worm burden

Mature adults were recovered from a wide range of host sizes. Larger hosts were relatively more frequently and heavily infected (Table 1). Increased food volume in older fish and their longer life-span allowing for cummulative infections might be involved.

Biacetabulum biloculoides Mackiewicz and McCrae, 1965

Distribution

This cestode was previously reported in the same host from Colorado, Iowa, New York, North Carolina, Ohio, Pennsylvania, South Carolina, and South Dakota by Mackiewicz and McCrae (1965). The present report from Pike River white suckers represents a new species record in Wisconsin and considerably extends its known range of distribution. Some of the specimens labeled "*Glaridacris catostomi*" (by other authors) from Iowa, New York, and South Dakota were redetermined by Mackiewicz and McCrae as *B. biloculoides*. Whether some of the *G. catostomi* previously recorded elsewhere in Wisconsin were actually *B. biloculoides* remains to be determined.

B. biloculoides infected Pike River suckers less heavily (20% infection rate, mean 0.4, range 1–4 per host) (Table 1) than it infected hosts in New York (mean 5.8, range 1–37 per host) and in North Carolina (maximum of 46 worms in one host) (Mackiewicz and McCrae, 1965).

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Structural observations

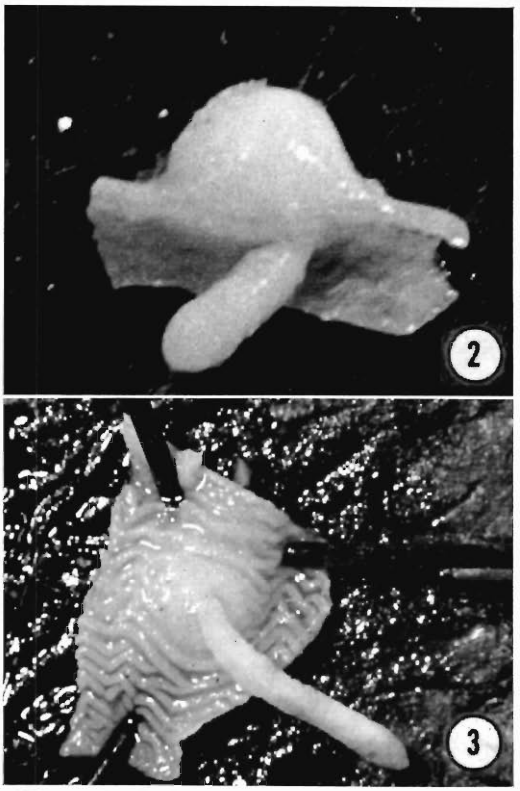
A total of 50 adult worms was recovered, five of which were young adults. All worms were more variable in shape, relatively shorter and broader, and occasionally with a relatively more pronounced scolex (Fig. 1C) than those described by Mackiewicz and McCrae from Colorado and New York. Whole mounts of mature adults were 2.00–6.80 (mean 4.32) in total length, 0.52–1.76 (mean 1.04) in maximum width (at male gonopore), 0.44–0.84 (mean 0.56) in scolex width, and 0.24–0.60 (mean 0.44) in neck width ($N = 17$). Corresponding measurements of my young adults were 1.12–1.88 (mean 1.41), 0.36–0.68 (mean 0.48), 0.24–0.56 (mean 0.36), and 0.16–0.44 (mean 0.28) ($N = 4$). Mackiewicz and McCrae's mature adults were 3.7–11 long and 0.4–1.0 wide and their young adults were 0.9–3.7 long.

Effect of host on worm burden

A comparison of infection rates (12% and 28%) and means per host (0.3 and 0.5) of *B. biloculoides* infections in Pike River suckers belonging to the first two host size classes (5–9.9 and 10–14.9 cm long) (Table 1) shows a positive correlation between increased host size and worm burden. Similar correlations were observed in other caryophyllaeid cestodes, v.g.; *Isoglaridacris hexacotyle*, which were related to the proportional increase in food volume (containing the intermediate host) in larger fish as well as to cumulative effect of parasitic infections in older (larger) fish (Amin, 1969). *B. biloculoides* was also observed to attain larger sizes in older hosts. Unmounted worms (young and mature) from 5–9.9- and 10–14.9-cm-long suckers were 1.0–6.0 (mean 3.5) ($N = 16$) and 1.0–7.0 (mean 4.7) ($N = 26$), respectively, in total length.

Effect of parasite on host and site of infection

All adults exclusively infected the stomach. The scolex and often the neck of most specimens was buried in relatively deep pits with elevated rims lacking the characteristic zigzag-like striation of the stomach mucosa. On the serosal side, these pits appeared as cysts, which were occasionally relatively large. No more than one specimen was found in a single pit (Figs. 2, 3). These observations vary somewhat from those of Mackiewicz and McCrae (1965) who reported their specimens in the stomach as well as in the small intestine, recovered up to six specimens from one pit, and indicated the pits as being "shallow." The fact that the maximum number of *B. biloculoides* per host from the Pike River was four while it reached 37 and 46 in New York and North Carolina, respectively (Mackiewicz and McCrae, 1965), might, as a result of crowding, account for the difference in the number of worms per pit indicated above.



Figures 2, 3. ***Biacetabulum biloculoides.*** **2. Side view of a young adult (1.5 mm long) with the whole scolex buried in a deep pit in the stomach mucosa of a white sucker. 3. View of a mature adult (5.0 mm long) with the scolex and part of the neck buried in another stomach pit of the same host; note the elevated smooth pit rim.**

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Acanthocephalus dirus (Van Cleave, 1931) Van Cleave and Townsend, 1936

Distribution

A. dirus was first reported from *Aplodinotus grunniens* in Mississippi by Van Cleave (1931). Subsequently, it was reported from the same host as well as from *Helioperca incisor*, *Huro salmoides*, and *Ictalurus punctatus* in Illinois by Van Cleave and Townsend (1936) and from *A. grunniens* in Tennessee by Bangham and Venard (1942). Its recovery from *C. commersoni* in Wisconsin represents a new host and state record.

Structural observations

The description of *A. dirus* by Van Cleave (1931) was very brief. It mainly included counts of proboscis hook rows and hooks and measurements of body and embryo dimensions and proboscis hook length. The lemnisci were about ⅔ the length of the proboscis receptacle (Van Cleave, 1931) but later observed to be "considerably longer" (Van Cleave and Townsend, 1936).

Many new significant observations and measurements are added herein, made on 146 specimens (67 males and 79 females, sex ratio 1 male:1.18 females). My adults were considerably smaller than those described by Van Cleave (his males and females were 4–6 and 10–20 mm long). The following two reasons are probably involved: (1) my specimens were young adults; none of the females contained embryos, only ovarian balls; (2) a number of my specimens was not fully relaxed, probably as a result of initial fixation in formalin in host intestines.

Sexual dimorphism was quite apparent; females were larger than males in size of the body, proboscis, proboscis hooks, proboscis receptacles, and lemnisci. In both sexes, the proboscis was cylindrical, occasionally slightly swollen, and typically directed ventrad. Lemnisci were usually elongate (occasionally bulboid or oval) with their posterior end between the anterior ⅓ and base of the double-walled proboscis receptacle. In a relaxed male, the hook-bearing part of the fully everted proboscis assumed an almost rectangular outline and was followed posteriorly by a narrow long cylindrical "neck."

In males, the reproductive system occupied the posterior ⅔ to ⅞ of the body. Variation in this character largely depended on the degree of contraction of specimens. Males had two usually contiguous testes, rarely one (2/67). The anterior testis was usually slightly larger than the posterior ones but smaller than the single testes in monorchid specimens. Anteriorly, the anterior testis usually extended beyond the base of the proboscis receptacle and not uncommonly was overlapped by the posterior portion of the lemnisci. The number of cement glands was six.

The following measurements (length by width) are of 32 whole mounts (13 males and 19 females) (mean figures are in parentheses):

MALES: Body (excluding proboscis): 1.20–2.96 (1.97) by 0.34–0.52 (0.44). Proboscis: 0.294–0.364 (0.336) by 0.126–0.154 (0.139). When fully everted, one proboscis was 0.196 long and 0.133 wide ("neck" excluded). Length of largest proboscis hooks: 0.042–0.056 (0.049). Proboscis receptacle: 0.308–0.686 (0.496) by 0.140–0.196 (0.154). Lemnisci: 0.182–0.238 (0.224) by 0.112–0.196 (0.158). Anterior testis: 0.364–0.658 (0.529) by 0.238–0.336 (0.288). Posterior testis: 0.322–0.700 (0.490) by 0.224–0.364 (0.272). A single testis in a monorchid male was 0.868 by 0.308. Cement glands: 0.126–0.252 (0.182) by 0.070–0.168 (0.102).

FEMALES: Body (excluding proboscis): 2.92–8.50 (4.36) by 0.40–0.72 (0.56). Proboscis: 0.378–0.532 (0.440) by 0.196–0.266 (0.224). Length of largest proboscis hooks: 0.045–0.070 (0.067). Proboscis receptacle: 0.448–0.840 (0.668) by 0.182–0.224 (0.203). Lemnisci: 0.196–0.322 (0.276) by 0.112–0.238 (0.164).

Infection load and site

Older hosts were obviously more heavily and frequently infected with *A. dirus* than younger ones. Infection rates and means were 100%, 37%, 14% and 13.2, 0.7, 0.5, in that order (Table 1). Increased food volume containing the intermediate host consumed by older hosts as well as the longer life-span of these hosts allowing for cumulative infections probably account for this pattern. Relative changes in diet composition might be involved. The proboscis of many worms was more or less deeply inserted in the intestinal wall, resulting in moderate to strong attachment of

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the worms, some of which were difficult to remove. No significant difference in the preferred site of infection (small intestine) was noted in the different host size classes. Of 32, 48, and 66 worms recovered from the three host size classes (Table 1), 6, 2, and 6% were recovered from the large intestine.

Dorylaimus sp. (?) Dujardin, 1845

Dorylaimids are free-living nematodes in the mud at the bottom of ponds, lakes, and streams as well as in moist soil. Species of *Dorylaimus* are known to feed on algae, mosses, plant roots, and other nematodes. The presence of the buccal spear (stylet) in nematodes is considered to be an adaptation to predaceous or parasitic life. This eversible structure was markedly conspicuous in my specimens, particularly when extended out of the mouth.

Dorylaimus sp. was previously reported from *Erimyzon sucetta oblongus*, *Eupomotis gibbosus*, *Cyprinus carpio*, and *Percopsis omiscomaycus* in Oneida Lake, New York, by Van Cleave and Mueller (1934). In the present study over 1,000 specimens were obtained from 36 infected *C. commersoni* (Table 1) which represents a new host and state record. The bottom-feeding habits of *C. commersoni* must account for this finding. The feeding grounds of the white sucker in the examined localities of the Pike River must be extremely rich with populations of this nematode. The phenomenon of "swarming" previously reported in *Dorylaimus* by McBride and Hollis (1966; in Croll, 1970) might be involved. The worms were found invariably suspended within the host's intestinal food mass or occasionally loose in the absence of significant volumes of food. Despite the significantly high "infection" rate, the present record must, however, be considered a case of accidental infection. The localization of worms referred to above partially supports this hypothesis.

A few hundred female worms only were observed. They were 3.80–6.00 long and 0.098–0.168 wide and they were found in the small intestine (59%), stomach (27%), and large intestine (14%). Mixing of sites was not uncommon. The lowest "infection" rate (16%) and mean (1.4 per host) were noted for the smallest hosts. The medium-size hosts had higher figures of 36% and 14.0 worms per host, corresponding with increased food intake in larger fish. The largest hosts, however, were free of nematodes (Table 1). A relative change in feeding habits of larger suckers (probably involving different strata of feeding grounds) as well as smaller sample size ($N = 5$) might account for this finding.

Conclusions

1) The degree of ecological isolation, as probably determined by the differential distribution of hosts of immatures of the above-reported helminths, was very apparent in this study. With the exception of *T. attenuatum*, all other worms were found in suckers taken in either the Root or Pike River, but not in both. Amin et al. (1973) also reported the copepod *Lernaea cyprinacea* from 10 fish species from the Root River, but from none of 11 fish species examined from the Pike River.

2) An increased worm burden by age was demonstrated for all but *T. attenuatum*.

3) Increased mixed infections also corresponded with age. Of 22 Pike River suckers with mixed infections, only two of the hosts were of the small size category.

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Helminth Parasites of the Brown Pelican in Florida and Louisiana[1]

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ABSTRACT: One hundred and thirteen brown pelicans collected from Florida and Louisiana were infected with 31 species of helminths including 14 trematodes, 4 cestodes, 11 nematodes, and 2 acanthocephalans.

Geographical location of the pelicans had a greater influence than age of the host upon helminth prevalence and intensity. More species were found in birds from the Atlantic coast of Florida (22) than from the Gulf coast of Florida (15) or Louisiana (12). There were more species in adults (22) and subadults (20) than birds-of-the-year (15).

The helminth fauna was almost complete in nestlings by the time they were fledged. Some helminths were present in nestlings as young as 2 weeks, and the geographical location of the colony appeared to influence the pattern of helminth acquisition.

In 1965 the Florida Game and Fresh Water Fish Commission began a long-term study of the brown pelican, *Pelecanus occidentalis*, with the following objectives: (1) censusing the nesting population of brown pelicans in Florida, (2) determining the causes of unusual mortality among pelicans, and (3) determining the threat posed to the population of brown pelicans by infectious diseases, parasites, and chemical pollution (Williams and Martin, 1968). As part of that project, the present study was conducted from 1971 to 1973 to gather information on the prevalence and intensity of helminth parasites and to determine differences in helminth burdens associated with age and geographical location of brown pelicans.

No systematic survey of the helminth parasites infecting brown pelicans has been reported. Various authors have examined small numbers of pelicans and reported 17 species of helminths. Ten trematodes have been reported: *Gigantobilharzia* sp. by Leigh (1957), *Renicola thapari* by Caballero (1953), *Paracoenogonimus ovatus* by Odening (1963), *Phagicola longus* by Price (1933) and Hutton and Sogandares-Bernal (1960), *Galactosomum darbyi* by Price (1934), *G. puffini* by Caballero et al. (1953), *Galactosomum* sp. by Hutton and Sogandares-Bernal (1960), *Mesostephanus appendiculatoides* by Price (1934), Caballero et al. (1953), and Hutton and Sogandares-Bernal (1960), *M. microbursa* by Caballero et al. (1953), and *M. yedeae* by Dennis (1968). Two cestodes have been reported: *Tetrabothrius sulae* by Flores-Barroeta et al. (1958) and *Paratetrabothrius orientalis* by Flores-Barroeta (1955). Five species of nematodes have been reported: *Syngamus* sp. and *Contracaecum* sp. by Vasquez and Chavez-Garcia (1962), *Contracaecum spiculigerum* by Huizinga (1971), and *Contracaecum mexicanum* and *Physaloptera maxillaris* by Flores-Barroeta (1957). There have been no reports of acanthocephalans from brown pelicans.

Materials and Methods

Fifty-seven fledged birds from widely scattered localities in peninsular Florida were collected by biologists of the Florida Game and Fresh Water Fish Commission and other cooperators. Nine nestlings were obtained from various colonies on the Atlantic coast of Florida and a series of 30 nestlings from Bird Key (Lee County) on the Gulf coast of Florida. Biologists of the Louisiana Wild Life and Fisheries Commission provided 14 pelicans which had been shot or found dead at Grande Terre, Louisiana. The Louisiana pelicans had been transplanted from the Atlantic coast of Florida as nestlings in 1968, 1969, and 1970.

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Table 1. Helminths of 113 brown pelicans from Florida and Louisiana.

Helminth	Per cent infected	Number of worms per infected bird	
		Mean	Range
TREMATODA			
Phagicola spp.* (3,4,5,6)	86.8	6,590	2–36,650
Mesostephanus appendiculatoides (3,4,5,6)‡‡	79.8	1,112	1–10,900
Stephanoprora denticulata† (3,4,5,6)	47.4	54	1–850
Renicola thapari (10)	43.8	108	1–1,500
Galactosomum darbyi (3,4,5,6)	36.0	89	1–1,000
Austrobilharzia sp.† (7)	18.4	3	1–12
Galactosomum fregatae (3,6)	8.8	3	1–8
Echinochasmus sp. like *dietzevi*‡ (3)	6.1	9	1–25
Carneophallus turgidus† (3,4)	3.5	178	1–650
Ascocotyle (*Leighia*) sp.‡ (3)	<1	80	–
Microparyphium facetum† (3)	<1	1	–
Pholeter sp.‡ (3)	<1	16	–
CESTODA			
Tetrabothrius sp. (3,6)	36.8	5	1–25
Parvitaenia ibisae† (3,5)	21.9	9	1–67
Parvitaenia heardi (3)	<1	2	–
Unidentified cestode fragments (3)	<1	1	–
NEMATODA			
Contracaecum spp.** (1,2,3)	91.3	59	1–1,192
Cosmocephalus obvelatus† (1,2)	40.0	4	1–35
Paracuaria tridentata† (1,2)	17.4	7	1–46
Cyathostoma phenisci† (8,9)	12.3	2	1–4
Capillaria sp. like *contorta*‡ (1,2)	10.5	2	1–4
Capillaria sp. like *mergi*‡ (3,4,6)	9.6	806	1–8,850
Unidentified schistorophid larva† (1,2)	1.8	20	10–30
Physaloptera sp. (3)	<1	1	–
Synhimantus invaginatus† (1)	<1	1	–
Tetrameres inerme† (2)	<1	1	–
ACANTHOCEPHALA			
Southwellina hispida† (3,4,5)	16.7	28	1–197
Corynosoma sp.† (3)	<1	2	–

Numbers in parentheses indicate site in host: (1)—esophagus; (2)—proventriculus; (3)—small intestine; (4)—ceca; (5)—large intestine; (6)—cloaca; (7)—blood vessels; (8) trachea; (9)—lungs; (10)—kidney.

* A complex of two species: *P. longus* and *Phagicola* sp. like *minutus*.‡

** A complex of two species: *C. spiculigerum* and *C. multipapillatum*.†

† New host records.

‡ Probably represents new species.

‡‡ A few birds contained small numbers of *M. microbursa* as well as *M. appendiculatoides*. Differential counts were not made.

Three pelicans that had been kept in captivity in Florida were also examined.

Procedures for recovering, killing, fixing, and studying helminths were similar to those described by Kinsella and Forrester (1972). Where flukes were very abundant, their numbers were determined by mixing the flukes thoroughly with 250 ml of water and counting a 10-ml aliquot.

Analysis of variance was used to determine if the prevalence and intensity data for a given helminth could be grouped by locality regardless of host age and grouped by host age regardless of the locality of capture. Where grouping was permissable, the significance of differences in prevalence and intensity of a helminth at any two localities or between any two age groups was determined by Student's *t* test.

Results and Discussion

Thirty-one species of helminths were recovered from 113 pelicans. Table 1 lists the species recovered, prevalence of infection, mean intensity of infection with ranges, and site of infection. Twenty-one are new host records.

Geographical pattern of helminth infections

Table 2 lists the prevalence and intensity of helminth infections in fledged pelicans from three general localities—the Atlantic and Gulf coasts of Florida, and Louisiana. Helminths found in nestlings, birds collected in the Florida Keys where Atlantic and Gulf populations intermingle, and captive pelicans were excluded from this analysis.

In general, more helminth species were

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Table 2. Prevalence and intensity of helminths of three populations of brown pelicans.

Helminth	Atlantic coast (39 birds)		Gulf coast (14 birds)		Louisiana (14 birds)	
	Number infected	Mean intensity	Number infected	Mean intensity	Number infected	Mean intensity
TREMATODA						
P. longus	39	9,058	14	3,677	14	11,849
M. appendiculatoides	31	785	14	1,224	2	1
S. denticulata	28	43	6	2	14	117
R. thapari	13	20	13	201	0	0
G. darbyi	8	174	12	56	1	1
Austrobilharzia sp.	21	3	0	0	0	0
G. fregatae	2	2	4	4	0	0
Echinochasmus sp.	4	12	0	0	0	0
C. turgidus	4	178	0	0	0	0
Ascocotyle sp.	1	80	0	0	0	0
M. facetum	1	1	0	0	0	0
CESTODA						
Tetrabothrius sp.	24	3	11	5	4	20
P. ibisae	14	7	5	12	3	3
NEMATODA						
Contracaecum spp.	37	22	14	66	12	25
C. obvelatus	28	3	5	8	3	1
P. tridentata	13	10	3	2	2	0
C. phenisci	10	2	0	0	0	0
Capillaria sp. like *contorta*	8	1	0	0	2	2
Capillaria sp. like *mergi*	11	806	0	0	0	0
Schistorophid larva	0	0	2	20	0	0
Physaloptera sp.	1	1	0	0	0	0
S. invaginatus	0	0	0	0	1	1
T. inerme	0	0	1	1	0	0
ACANTHOCEPHALA						
S. hispida	5	3	6	86	3	1
Corynosoma sp.	0	0	1	2	0	0

found in birds from the Atlantic coast of Florida (22) than the Gulf coast (15) or Louisiana (12). There were more significant differences in helminth prevalence between the Atlantic and Gulf coasts and between the Atlantic coast and Louisiana than between the Gulf coast and Louisiana. *Phagicola longus* occurred in all birds at all three localities and could not be analyzed statistically. Statistical analysis showed the following to be significant: *Stephanoprora denticulata* occurred most often in Louisiana birds and least often on the Gulf coast. *Renicola thapari* occurred most often on the Gulf coast and was absent altogether from Louisiana. *Tetrabothrius* sp. and *M. appendiculatoides* were least prevalent in Louisiana. *Galactosomum fregatae* and *G. darbyi* were most prevalent on the Gulf coast. *Cyathostoma phenisci*, *Austrobilharzia* sp., and *Capillaria* sp. like *C. mergi* occurred only on the Atlantic coast. *Southwellina hispida* was more prevalent on the Gulf coast than the Atlantic, but was not significantly more prevalent on the Gulf coast than in Louisiana. The remaining species either showed no significant difference in prevalence at the localities concerned or the sample size was too small to detect any such differences.

There were more significant differences in the intensity of infection between the Atlantic and Gulf coasts and the Gulf coast of Louisiana than between the Atlantic coast and Louisiana. Statistical analysis showed the following to be significant: *Contracaecum* spp., *M. appendiculatoides*, *R. thapari*, and *S. hispida* were all most abundant in infections from the Gulf coast, whereas *P. longus* was least abundant there. *Tetrabothrius* sp. and *S. denticulata* were most abundant in Louisiana birds.

The influence of host age on helminth infections

Table 3 lists the prevalence and intensity of helminth infections by age of the pelican hosts. The following groupings were used. "Birds-of-the-year" were all birds less than 1 year of age excluding nestlings. "Subadults" included all birds in their 2nd year and 3rd year.

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Table 3. Prevalence and intensity of helminths of three age classes of brown pelicans.

Helminth	Adults (20 birds)		Subadults (36 birds)		Birds-of-year (11 birds)	
	Number infected	Mean intensity	Number infected	Mean intensity	Number infected	Mean intensity
TREMATODA						
P. longus	20	5,994	36	9,025	11	11,430
M. appendiculatoides	15	1,133	25	737	7	690
S. denticulata	14	31	27	43	7	183
R. thapari	8	70	13	157	5	55
G. darbyi	5	47	13	119	3	39
A. terrigalensis	3	5	15	3	3	1
G. fregatae	5	3	2	4	0	0
Echinochasmus sp.	2	9	2	15	0	0
C turgidus	1	2	3	237	0	0
Ascocotyle sp.	1	80	0	0	0	0
M. facetum	1	1	0	0	0	0
CESTODA						
Tetrabothrius sp.	9	7	20	4	8	6
P. ibisae	4	20	12	7	6	3
NEMATODA						
Contracaecum spp.	18	28	34	37	11	23
C. obvelatus	8	5	23	4	5	2
P. tridentata	7	2	9	13	2	2
C. phenisci	2	2	6	2	3	3
Capillaria sp. like *contorta*	2	2	8	1	0	0
Capillaria sp. like *mergi*	2	2	8	1,108	1	2
Schistorophid larva	2	20	0	0	0	0
Physaloptera sp.	0	0	0	0	1	1
S. invaginatus	1	1	0	0	0	0
T. inerme	0	0	1	1	0	0
ACANTHOCEPHALA						
S. hispida	8	41	3	67	2	2
Corynosoma sp.	0	0	1	2	0	0

"Adults" were birds 3 years or older. Birds from the Florida Keys and captives were not included in this analysis.

In general there were fewer differences in helminth prevalence due to age than due to locality. More species were found in adults (22) and subadults (20) than birds-of-the-year (15). *Phagicola longus* occurred in all birds of all ages. Statistical analysis showed the following to be significant: *Galactosomum fregatae* was most prevalent in adults. *Southwellina hispida* was more prevalent in adults than subadults but not birds-of-the-year. *Capillaria* sp. like *C. contorta* was more prevalent in subadults than birds-of-the-year but not adults. *Austrobilharzia* sp. was more prevalent in subadults than adults but not birds-of-the-year.

There was no general pattern of helminth intensity by host age. *Stephanoprora denticulata* was most abundant in birds-of-the-year, and *P. ibisae* was most abundant in adults. *Phagicola longus* was more abundant in birds-of-the-year than adults but not subadults. *Paracuaria tridentata* was more abundant in subadults than adults but not birds-of-the-year.

Helminth acquisition by nestlings

Five age classes of nestlings (2, 3, 4½, 7, and 9 weeks of age) from the Bird Key Colony on the Gulf coast of Florida and three age classes (4½, 7, and 9) from various colonies on the Atlantic coast were examined to determine the pattern of helminth acquisition by nestling pelicans.

On the Gulf coast, *M. appendiculatoides*, *R. thapari*, *G. darbyi*, and *Contracaecum* spp. appeared in birds as young as 2 weeks, the youngest age class examined. No new helminth species appeared until 4½ weeks when *P. longus*, *Phagicola* sp., *S. denticulata*, *G. fregatae*, *Parvitaenia heardi*, and *S. hispida* were found. At 7 weeks *Echinochasmus* sp. appeared, and by 9 weeks *C. obvelatus* and *Tetrabothrius* sp. were present. Generally, the intensity of infections increased with host age.

On the Atlantic coast, *Echinochasmus* sp., *C. obvelatus*, *Pholeter* sp., and *C. phenisci* were present in 4½-week-old birds whereas *R. thapari*, *P. ibisae*, and *P. tridentata* appeared at 9 weeks.

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Significance of the helminth fauna to the host

Because of their relatively great prevalence and intensity in brown pelicans, four helminths (*Contracaecum* spp., *P. longus*, and *M. appendiculatoides*) may be potential pathogens. Species of the same or related genera have been implicated as pathogens in aquatic birds (Willey and Stunkard, 1942; Huizinga, 1971; Liu and Edward, 1971; Gibson et al., 1972).

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Brachylaimid and Dicrocoeliid Trematodes of Birds from North Borneo (Malaysia)[1]

JACOB H. FISCHTHAL AND ROBERT E. KUNTZ[2]

ABSTRACT: One brachylaimid and 10 dicrocoeliid trematodes of birds are reported from North Borneo (Malaysia). New species described are *Brachylaima* (*Brachylaima*) *sabahense*, *Brachydistomum api*, *Brachylecithum pycnonoti*, *B. sabahense*, *B. vitellobum*, and *Lyperosomum malaysiae*. Briefly described are *Brachylecithum attenuatum* (Dujardin, 1845) Shtrom, 1940, *Lutztrema bhattacharyai* (Pande, 1939) Travassos, 1944, and *Proacetabulorchis dogieli* Belopolskaja and Bykhovskaja-Pavlovskaja, 1953. Also reported are *Athesmia heterolecithodes* (Braun, 1899) Looss, 1899, and *Proacetabulorchis prashadi* Gogate, 1940.

The trematodes of this paper are part of a collection made by the junior author while a member of the U. S. Naval Medical Research Unit No. 2, Taipei, Taiwan. Parasites were washed in saline, killed in hot water, and transferred immediately to FAA fixative; after 4 to 8 hr they were stored in 70% alcohol plus 2% glycerin. Staining was in Mayer's carmalum, and all were mounted in Permount. Host names recorded herein are those listed by Kuntz (1969). Host names preceded by an asterisk (*) represent new host records. Specimens of each trematode species reported have been deposited in the U. S. National Museum Helminthological Collection as noted. All measurements are in microns.

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Family Brachylaimidae

Brachylaima (*Brachylaima*) *sabahense* sp. n.

(Figs. 1, 2)

HOSTS: Type, *Aplonis panayensis* (Scopali), starling (Passeriformes: Sturnidae); *Orthotomus sepium borncoensis* Salvadori, red-headed tailor bird (Passeriformes: Muscicapidae: Sylvinae); *Nyctyornis amictus* (Temminck), red-bearded bee-eater (Coraciiformes: Meropidae).

HABITAT: Small intestine.

LOCALITIES: Kasiqui, Petergas.

DATES: 3, 16 September 1960.

SPECIMENS DEPOSITED: No. 72713 (holotype, from *Aplonis*); No. 72714 (paratypes, *Orthotomus*); No. 72715 (paratype, *Nyctyornis*).

DIAGNOSIS (based on nine adult worms: one in ventral view from *Aplonis*, one from *Nyctyornis* and seven from *Orthotomus* in lateral view; measurements of one from each host species are length by width by depth): Body elongate, gonadal region narrower than remainder of body, widest at acetabular level, extremities rounded, 955–1,970 by 360 by 445–520, tegument spined dorsally to level between pharynx and acetabulum in extended worms and ventrally to latter or slightly more posteriorly. Forebody 255–550 long; hindbody 550–1,240 long; forebody–hindbody length ratio 1:1.67–2.25. Oral sucker ventral, longitudinally elongate, with narrow longitudinal aperture, 170–210 by 153 by 186–205; preoral space 22–55 long. Acetabulum round, 150–180 by 150 by 162–174. Sucker length ratio

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1:0.79–0.88, width ratio 1:0.98, depth ratio 1:0.85–0.87. Prepharynx present, usually not discernible, 24 long in one worm in lateral view; pharynx 90–145 by 115 by 126–140; esophagus very short, obscured by eggs; ceca narrow, cell-lined, terminating just posttesticular near posterior extremity.

Testes two, diagonal, smooth, separated or contiguous; anterior testis usually dextral, occasionally sinistral, overlapping cecum, 153–345 by 148 by 242–262, lying 148–320 postacetabular; posterior testis median, embraced by ceca, smaller to larger than anterior testis, 126–320 by 125 by 203–242; posttesticular space 65–215 long. External seminal vesicle coiled anterior to anterior testis, partly covered by uterine coils, tubular. Pars prostatica short, surrounded by few prostate cells. Cirrus sac muscular, 104–195 by 73 by 48–109, lying median to anterior half of anterior testis to being entirely pretesticular, containing muscular, winding, unspined cirrus. Genital pore ventral to cirrus sac.

Ovary smooth, dorsomedian, in tandem or nearly so with posterior testis, smaller than testes, 102–208 by 115 by 135–153, overlapping testes dorsally. Mehlis' gland well developed, ventral to ovary. Vitelline follicles extending from oral sucker, pharyngeal or just postpharyngeal level to anterior half of anterior testis, lying dorsal, lateral, and ventral to ceca, few follicles invading slightly intercecal space. Vitelline ducts descending posteromedianly from posterior end of each vitelline field, uniting ventral to ovary to form reservoir. Uterine coils mainly pretesticular, filling intercecal space, overlapping ceca, filling prececal space to level of oral sucker, some coils lying dorsal to latter. Metraterm thick-walled, muscular, surrounded by gland cells, entering genital atrium near cirrus opening. Eggs numerous, yellow-brown, operculate, 15 measuring 23–30 (25.1) by 15–18 (16.3).

Excretory bladder short, saccular, entirely posttesticular; pore subterminal ventral.

Discussion

Six of the specimens from the tailor bird were too contracted for adequate measurements. Our new species is closest to those species of the subgenus from birds listed by Yamaguti (1971) with the vitellaria extending into the forebody: *B. centrodes* (Braun, 1901) Dollfus, 1935 (Brazil); *B. columbae* (Mazzanti, 1889) Dollfus, 1934 (Italy); *B. degiustii* Nasir and Rodriguez, 1966 (Venezuela); *B. marsupium* (Braun, 1901) Dollfus, 1935 (Brazil); *B. mazzantii* (Travassos, 1927) Dollfus, 1935 (Brazil, Panama, Kirgiz SSR); *B. syrmatici* (Yamaguti, 1935) Yamaguti, 1939 (Japan); *B. tisa* Chatterji, 1956 (India). All these species differ from ours in being larger and the uterine coils not extending prececally. *B. centrodes* differs further in having the acetabulum more posteriorly situated and a spined cirrus; *B. columbae* in the acetabulum being much smaller than the oral sucker and the vitellaria commencing just preacetabular; *B. degiustii* in being spined to at least the posterior testis level, and having a round oral sucker aperture, a very long hindbody, and tandem gonads; *B. marsupium* in having a muscular pad just anterior to the longitudinally oval oral sucker aperture; *B. mazzantii* in having a transversely oval oral sucker aperture, a very long hindbody, tandem testes, and a rudimentary cirrus sac; *B. syrmatici* in having an unspined tegument and a nearly equatorial acetabulum; and *B. tisa* in having an unspined tegument and a rudimentary (?) cirrus sac, and the vitellaria commencing just preacetabular.

Family Dicrocoeliidae

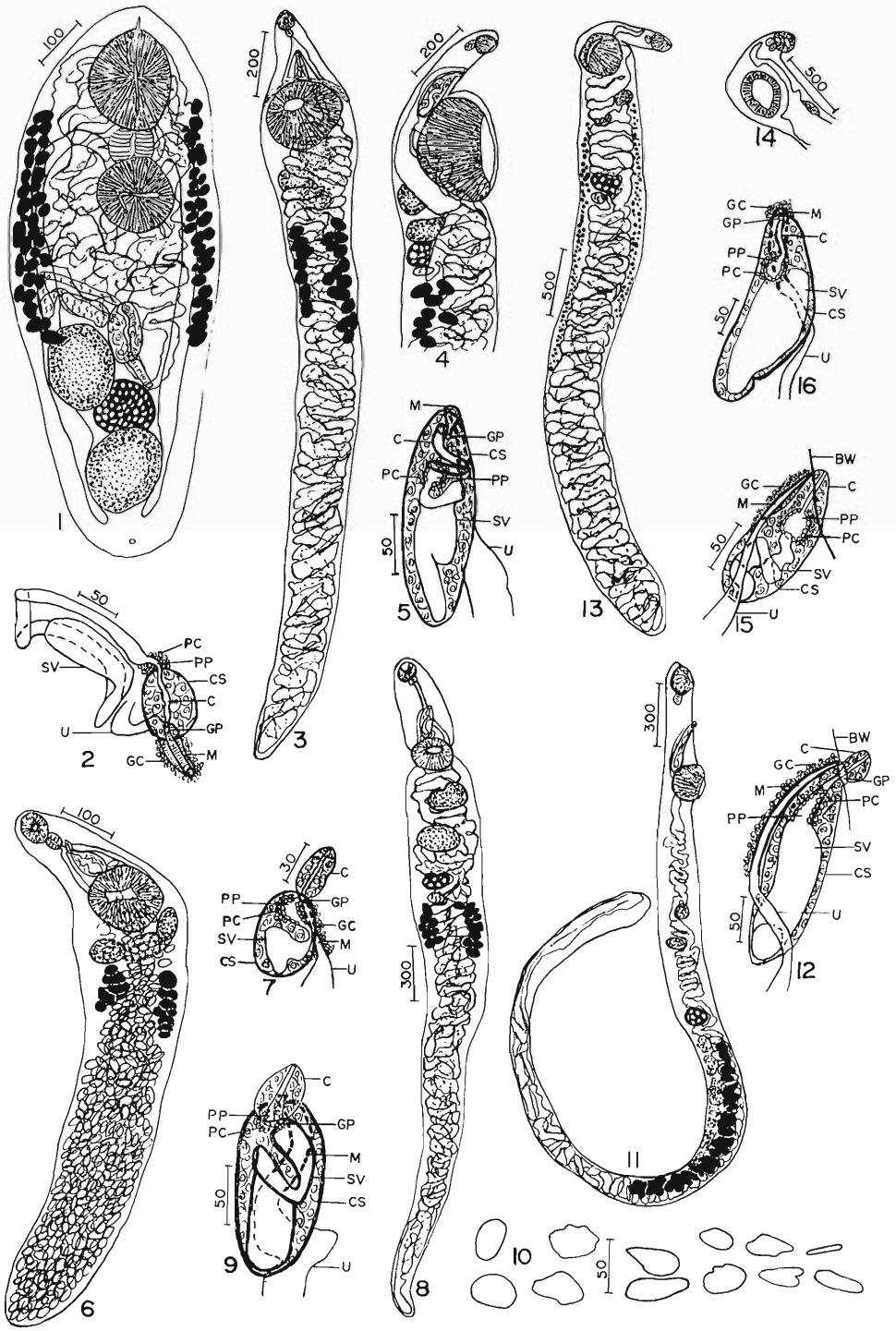
Athesmia heterolecithodes (Braun, 1899) Looss, 1899

HOST: **Amaurornis phoenicurus javanicus* (Horsfield), water hen (Gruiformes: Rallidae).
HABITAT: Liver.
LOCALITIES: Inanam, Ranau.
DATES: 1, 24 September 1960.
SPECIMENS DEPOSITED: No. 72716.

Discussion

Fragments of one worm from one host and two from another were in our collection. Hammond (1972) presented an historical review of the genus, noting the great variation occurring in individuals from different host species, from different individuals of the same host species, and even in a population from the same host individual. The single worm from Inanam had smooth, elongate oval testes as in *A. pricei* McIntosh, 1937, and *A. kassimovi* Feizullaev, 1961; the ovary is slightly lobed

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as in the former species rather than smooth as in the latter; the ovary is smaller than either testis as in the latter species rather than larger as in the former. The two worms from Ranau have lobed gonads with the ovary larger than either testis as in *A. pricei*. Both *A. pricei* and *A. kassimovi* probably are synonyms of *A. heterolecithodes*.

Brachydistomum api sp. n. (Figs. 3–5)

HOST: *Apus affinis subfurcatus* (Blyth), house swift (Apodiformes: Apodidae).

HABITAT: Gall bladder.

LOCALITY: Kasiqui.

DATE: 30 August 1960.

SPECIMENS DEPOSITED: No. 72717 (holotype); No. 72718 (paratypes).

DIAGNOSIS (based on 11 adult worms from one swift; three complete worms in ventral view measured): Body elongate, narrow, widest at acetabular level, tapering to rounded extremities, with body fold anterior and anterolateral to acetabulum, 2,285–2,400 long by 300–350 wide. Forebody dorsoventrally flattened, dorsal in position compared to body just posteriorly, sharply demarcated from latter as seen in lateral view, curving slightly ventrally, 225–270 long. Hindbody somewhat rounded, 1,790–1,860 long. Forebody–hindbody length ratio 1:6.6–8.1. Oral sucker ventral, 68–73 by 73–85; preoral space 12–15 long. Acetabulum very large, nearly filling body width, rounded, 225–275 by 251–285. Sucker length ratio 1:3.31–3.82, width ratio 1:3.18–3.48. Prepharynx absent; pharynx 45–53 by 37–45, overlapping oral sucker dorsally; esophagus short; cecal bifurcation closer to pharynx than acetabulum; posterior extent of ceca obscured by eggs.

Testes two, smooth, tandem or oblique with anterior testis dextral or sinistral to posterior one, contiguous or nearly so, margins usually obscured by eggs; anterior testis overlapping acetabulum to entirely postacetabular, usually larger than posterior testis, occasionally about same size, 212 by 275 (in one); posterior testis median to submedian, 110–121 by 188–205 (in two). Cirrus sac relatively thin-walled, straight to slightly curved, 175–230 by 58–85, commencing 73–104 posterior to anterior margin of acetabulum. Seminal vesicle thin-walled, winding, longitudinal extent 90–147, nearly filling posterior two- to three-fifths of cirrus sac. Prostatic vesicle small, entirely anterior to seminal vesicle to completely overlapping anteriormost part, surrounded by few prostate cells. Cirrus muscular, undulating, protrusible. Genital pore aperture transverse, bifurcal or just postbifurcal, 62–72 posterior to oral sucker, 80–123 preacetabular.

Ovary smooth, transversely elongate, contiguous with posterior testis, overlapping latter or not, in tandem with posterior testis or with anterior testis, 110 by 162 (in one). Seminal receptacle postovarian, smaller than ovary. Vitellaria in lateral fields, just postovarian, one field longer than other, shorter field 235–385 long, longer field 350–445 long; vitelline reservoir posterior to seminal receptacle; postvitelline space 1,125–1,310 long. Uterus extensive, filling most of hindbody, passing anteriorly ventral to gonads, also much coiled dorsal to acetabulum, lying dorsal to cirrus sac. Metraterm short, commencing at level of anterior part of seminal vesicle, thick-walled, muscular, surrounded by few gland

←

Figures 1–16. ***Brachylaima* (*Brachylaima*) *sabahense*** **sp. n. 1. Whole mount, holotype, ventral view. 2. Terminal genitalia, holotype.** ***Brachydistomum api*** **sp. n. 3. Whole mount, holotype, ventral view. 4. Anterior end of body, paratype, dextrolateral view. 5. Terminal genitalia, holotype.** ***Brachylecithum pycnonoti*** **sp. n. 6. Whole mount, holotype, ventral view. 7. Terminal genitalia, holotype.** ***Brachylecithum sabahense*** **sp. n. 8. Whole mount, holotype, ventral view. 9. Terminal genitalia, holotype. 10. Testes of six paratypes showing variability in position, size, and shape.** ***Brachylecithum vitellobum*** **sp. n. 11. Whole mount, holotype, dextrolateral view. 12. Terminal genitalia, holotype.** ***Lyperosomum malaysiae*** **sp. n. 13. Whole mount, holotype, forebody in sinistrolateral view, remainder of body in ventral view. 14. Anterior end of body, paratype, ventral view. 15. Terminal genitalia, paratype, dorsodextral view.** ***Proacetabulorchis dogieli.*** **16. Terminal genitalia, ventral view.**

Abbreviations: BW, body wall; C, cirrus; CS, cirrus sac; GC, gland cells; GP, genital pore; M, metraterm; PC, prostate cells; PP, pars prostatica; SV, seminal vesicle; U, uterus.

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cells. Eggs numerous, thick-shelled, operculate, yellow-brown in proximal uterine coils, brown in distal coils, 15 measuring 30–34 (32) by 17–22 (20).

Excretory bladder long, tubular, commencing at level of seminal receptacle; pore terminal.

Discussion

Only three worms were measured as the others were either mounted in lateral view, broken, or incomplete. Two species of *Brachydistomum* Travassos, 1944, have been reported from the same host genus: *B. olssoni* (Railliet, 1900) Yamaguti, 1958 (Europe, Kirgiz SSR, Morocco); *B. salebrosum* (Braun, 1901) Travassos, 1944 (Europe, Siberian maritime region). Our species is closest to the latter but differs from it and all others in the genus, including *B. microscelis* (Yamaguti, 1933) Travassos, 1944 (Japan, India) and *B. longum* Oshmarin, 1970 (Vietnam), in having a much narrower, more attenuated hindbody and a much greater forebody–hindbody length ratio, and in the flattened part of the forebody being sharply demarcated from the body just posteriorly when seen in lateral view.

Brachylecithum attenuatum (Dujardin, 1845) Shtrom, 1940

HOST: **Pycnonotus zeylanicus* (Gmelin), yellow-crowned bulbul (Passeriformes: Pycnonotidae).

HABITAT: Small intestine.

LOCALITY: Kasiqui.

DATE: 1 September 1960.

SPECIMEN DEPOSITED: No. 72719.

DESCRIPTION (based on one adult worm in dextrolateral view; measurements are length by depth): Body 2,450 by 194 at acetabular level; forebody 385 long, hindbody 1,890 long, forebody–hindbody length ratio 1:4.9; oral sucker 109 by 83, with compact muscle layer within posterior, posterodorsal, and posteroventral margins; preoral space 5 long; acetabulum 175 by 162; sucker length ratio 1:1.61, depth ratio 1:1.95; pharynx 56 by 46; esophagus 67 long; extent of ceca obscured by eggs; gonads smooth, contiguous; anterior testis somewhat sinistral, 175 by 145, lying 95 postacetabular; posterior testis somewhat dextral, 177 by 162, slightly overlapping anterior testis level; cirrus sac thin-walled, 177 by 46, overlapping acetabulum 12; seminal vesicle winding; genital pore just postbifurcal; ovary somewhat sinistral, 121 by 138; seminal receptacle postovarian, contiguous with latter, diameter 72; vitelline fields postovarian, follicles smooth, right field 220 long, left 347 long, latter distance 14.2% of body length; postvitelline space 960 long, distance 39.2% of body length; uterus filling most of hindbody, with coil overlapping ovary–posterior testis contiguity, ascending over contiguity of testes, several coils between anterior testis and acetabulum; metraterm thick-walled, muscular, shorter than cirrus sac, dorsal to latter; eggs brownish, operculate, 10 measuring 29–33 (31) by 18–22 (20).

Discussion

This species has been reported from a variety of passeriform (Corvidae, Fringillidae, Hirundinidae, Motacillidae, Ploceidae, Turdidae) and piciform (Picidae) birds from Europe, Kirgiz and Tadzhik SSR, eastern maritime Siberia, and Japan.

Brachylecithum pycnonoti sp. n. (Figs. 6, 7)

HOST: *Pycnonotus goiavier gourdini* Gray, yellow-vented bulbul (Passeriformes: Pycnonotidae).

HABITAT: Gall bladder.

LOCALITY: Kapayan.

DATE: 26 September 1960.

SPECIMENS DEPOSITED: No. 72720 (holotype); No. 72721 (paratypes).

DIAGNOSIS (based on six adult worms; measurements are of holotype only): Body elongate, narrow, widest at or near acetabular level, 1,060 long by 207 wide. Forebody 180 long; hindbody 750 long; forebody–hindbody length ratio 1:4.2. Oral sucker subterminal ventral, round or nearly so, diameter 58, aperture transversely elongate; small preoral lobe usually present. Acetabulum round to transversely elongate, 130 by 145, aperture transversely elongate; sucker length ratio 1:2.24, width ratio 1:2.41. Prepharynx absent; pharynx round or nearly so, diameter 38; esophagus 12 long; cecal bifurcation closer to oral sucker than acetabulum; extent of ceca obscured by eggs.

Testes two, ovoid, smooth, more or less symmetrical at posterior margin of acetabulum; overlapping, contiguous, or separated from each

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other and from acetabulum; long axis obliquely or transversely oriented; anterior testis 58 by 85, posterior 56 by 80. Cirrus sac preacetabular, muscular, slightly thick-walled, 67 by 41. Seminal vesicle undulating, filling most of cirrus sac. Prostatic vesicle lying anterior to seminal vesicle, surrounded by few prostate cells. Cirrus muscular, protrusible. Genital pore slightly submedian, just postbifurcal.

Ovary smooth, transversely elongate, median to submedian, usually posttesticular, same size to larger than testes, 60 by 90. Seminal vesicle postovarian, contiguous with or separated from ovary. Mehlis' gland well developed, large, postovarian. Vitellaria postovarian, in lateral fields, considerably invading intercecal space, one field usually longer than other, maximum field length 133. Uterus filling hindbody posterior to vitellaria, usually with fewer anterior coils extending dorsal to ovary and acetabulum. Metraterm thick-walled, muscular, shorter than cirrus sac, surrounded by gland cells. Eggs numerous, brownish, thick-shelled, operculate, five measuring 35–36 by 22–24.

COMPOSITE MEASUREMENTS (LENGTH BY WIDTH BY DEPTH) OF ALL WORMS: Body 1,060 by 200–207 by 172; forebody 180 long, hindbody 750–2,005 long, forebody–hindbody length ratio 1:4.2; oral sucker 43–58 by 37–58 by 41–48; acetabulum 97–154 by 111–160 by 120; sucker length ratio 1:2.26–3.34, width ratio 1:2.41–3.00, depth ratio 1:2.95; pharynx 25–38 by 38 by 21–23; esophagus 12 long; right testis 45–80 by 73–102 by 82, left 51–80 by 73–100 by 90; cirrus sac 67 by 41; ovary 60–80 by 85–102 by 82; seminal receptacle 36–60 by 40–52 by 68; vitelline fields, maximum length 133–254; 20 eggs measuring 33–38 (35.5) by 21–24 (22.5).

Discussion

Only the holotype was suitable for complete measurements. Some, but not all, measurements were obtainable from the other worms as they were either broken, incomplete, or the forebody was distorted. Of those species of *Brachylecithum* Shtrom, 1940, from birds listed by Yamaguti (1971) our new species appears closest to *B. megacotyle* (Baer, 1959) Yamaguti, 1971, from apodiform (Apodidae) birds from Belgian Congo, and *B. platynosomoides* Potekhina, 1948, from passeriform (Paridae) birds from Kirgiz SSR. Although only one of our worms (hindbody 2,005 long, acetabulum 150 long) approaches the length of the latter species (2,975), its dimensions for the suckers, pharynx, gonads, and vitelline fields are considerably less. *B. platynosomoides* differs further in having a smaller sucker ratio (1:1.37) and larger eggs (48 by 30). Although most of our worms would probably fall within the length range of *B. megacotyle*, the dimensions of the suckers, pharynx, and cirrus sac for our species are considerably less. *B. megacotyle* differs further in having a wider body (300–365), an ovary smaller than the testes, and much shorter vitelline fields.

Brachylecithum sabahense sp. n. (Figs. 8–10)

HOST: *Halcyon chloris* (Boddaert), white-collared kingfisher (Coraciiformes: Alcedinidae).

HABITAT: Liver.

LOCALITIES: Tuaran, Petergas.

DATES: 9, 15 September 1960.

SPECIMENS DEPOSITED: No. 72722 (holotype); No. 72723 (paratypes).

DIAGNOSIS (based on three entire adult worms, two with posterior tip of body missing, and fragments of at least 31 others; first five measured): Body elongate, narrow, with constriction at acetabular level, widest at testicular level, tapering posteriorly to rounded point, 3,170–3,688 long by 360–460 wide. Forebody 390–460 long; hindbody 2,485–3,030 long; forebody–hindbody length ratio 1:6.4–6.6. Oral sucker round to longitudinally or transversely elongate, posteriorly longer ventrally than dorsally, with posterior concavity, wide muscle band within posterior and posterolateral margins in ventral view and dorsal, posterior and posteroventral margins in lateral view, 145–166 by 133–160; preoral space 0–28 long. Acetabulum wider than body at same level, transversely elongate, each lateral side rounded to somewhat pointed as for *B. eophonae* (Yamaguti, 1941) Skrjabin and Evranova, 1953, 198–245 by 245–295. Sucker length ratio 1:1.19–1.65, width ratio 1:1.56–1.88. Prepharynx absent; pharynx pyriform, partly within posterior concavity of oral sucker, 52–67 by 46–54; esophagus 102–174 long; cecal bifurcation closer to acetabulum than oral sucker; ceca narrow, conspicuously cell-lined, extending

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260–565 postvitellarian, terminating 1,265–1,415 from posterior extremity, latter distances 38–40% of body length.

Testes two, smooth to lobed, tandem to somewhat diagonal, transversely elongate, separated entirely or at least in part from each other, from acetabulum and from ovary by uterine coils; anterior testis 126–145 by 225–265, lying 75–120 postacetabular; posterior testis 133–157 by 218–263, lying 56–92 posterior to anterior testis. Cirrus sac elongate oval, thick-walled (up to 8), muscular, commencing at anterior margin of acetabulum or short distance posterior to margin, terminating at cecal bifurcation or just prebifurcal, 116–143 by 51–75. Seminal vesicle thick-walled, muscular, winding, filling most of cirrus sac. Prostatic vesicle surrounded by few prostate cells. Cirrus muscular, protrusible. Genital pore median, at bifurcal or just prebifurcal.

Ovary smooth, submedian to median, transversely elongate, 103–111 by 131–145, contiguous with posterior testis in one worm, lying 60–133 posttesticular in others. Seminal receptacle postovarian, contiguous with or separated from latter, 56–87 by 87–114. Mehlis' gland postovarian, median, well developed, within beginning of vitelline fields. Latter postovarian, one field longer than other, maximum length of fields 325–620, number of follicles (right–left) in five worms 9–11, 9–12, 10–9, 12–8, 12–9; postvitelline space 1,580–1,980 long, distances 49–54% of body length. Uterus filling postvitelline space, also coiling between and overlapping vitelline fields, ascending to one side of ovary, sending coils between ovary and posterior testis before returning to same side of body, passing between testes to opposite side of body, coils dorsal to acetabulum. Metraterm thick-walled, muscular, shorter than cirrus sac, ascending dorsal to latter, surrounded by few gland cells. Eggs numerous, brownish, operculate, 20 measuring 32–40 (35.4) by 16–22 (19.3).

Excretory bladder long, tubular, usually commencing at some level between posteriormost part of vitellaria and cecal ends, commencing short distance postcecally in one worm; pore terminal.

Discussion

Our host harbored four adult worms and another at least 32. Our new species is closest to *B. halcyonis* (Yamaguti, 1941) Skrjabin and Evranova, 1953, *B. uigurica* Evranova in Skrjabin and Evranova, 1953, and *B. palawanense* Fischthal and Kuntz, 1973. These species differ from the present one in lacking a constriction of the body at the acetabular level, and in having an acetabulum narrower than the body width at its level and an oral sucker of different shape and structure. *B. halcyonis* differs further in having round gonads and a pyriform, thin-walled cirrus sac; *B. uigurica* in having a round pharynx and a thin-walled cirrus sac, and the uterus not passing from one side of the body to the other between the testes; and *B. palawanense* in having a thin-walled seminal vesicle.

Brachylecithum vitellobum sp. n. (Figs. 11, 12)

HOST: *Amaurornis phoenicurus javanicus* (Horsfield), water hen (Gruiformes: Rallidae).

HABITAT: Small intestine.

LOCALITY: Petergas.

DATE: 15 September 1960.

SPECIMEN DEPOSITED: No. 72724 (holotype).

DIAGNOSIS (based on one adult worm in dextrolateral view; measurements are length by depth): Body elongate, narrow, deepest between acetabulum and anterior testis, 4,685 by 240. Forebody 500 long; hindbody 3,995 long; forebody–hindbody length ratio 1:8. Oral sucker ventral, with thick muscle band within posterodorsal, posterior, and posteroventral margins, 170 by 130; preoral space 17 long. Acetabulum 190 by 155, lateral elongations absent. Sucker length ratio 1:1.12, depth ratio 1:1.19. Prepharynx absent; pharynx deeper posteriorly than anteriorly, 51 by 51; esophagus 75 long; cecal bifurcation closer to acetabulum than oral sucker; posterior extent of ceca obscured by eggs.

Testes two, smooth, oblique, 80 apart; anterior testis dextral, 74 by 92, lying 495 postacetabular; posterior testis sinistral, 74 by 100. Cirrus sac elongate oval, thin-walled, commencing dorsal to anterior part of acetabulum, 235 by 65. Seminal vesicle saccular, filling most of cirrus sac. Prostatic vesicle surrounded by few prostate cells. Cirrus muscular, protrusible.

Ovary smooth, median, slightly larger than testes, 92 by 110, lying 275 posttesticular. Seminal receptacle postovarian, contiguous

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with latter, 40 by 57. Vitelline fields postovarian, very long, follicles very large, much lobed, longest field 1,000 (21.3% of body length). Uterus filling most of hindbody, six coils between ovary and posterior testis, passing between testes. Metraterm shorter than cirrus sac, thick-walled, ascending dorsal to cirrus sac, surrounded by gland cells. Eggs numerous, yellow-brown, operculate, 10 measuring 32–41 (36.1) by 17–21 (19.4).

Discussion

Our species appears closest to *B. filiforme biologicum* (Semenov, 1927) Shtrom, 1940, from a passeriform (Fringillidae) bird from Russia, *B. philippinense* Fischthal and Kuntz, 1973, from a passeriform (Muscicapidae) bird from Palawan Island, and *B. stunkardi* (Pande, 1939) Denton and Byrd, 1951, from passeriform (Corvidae) birds from India and United States, differing from them in having lobed rather than smooth vitelline follicles. *B. stunkardi* differs further from ours in having lateral appendages on the acetabulum, the anterior testis much closer to the acetabulum, the ovary close to the posterior testis and separated from it by one uterine coil, and a relatively shorter postvitelline space. *B. filiforme biologicum* differs further in having much longer gonads even though it is a smaller worm, tandem testes, the ovary relatively much closer to the posterior testis, and shorter vitelline fields (15% of body length). *B. philippinense* differs further in being a smaller worm, in the eggs averaging larger (43.1 by 23.2), and in having a smaller forebody–hindbody length ratio (1:4.8), the testes longitudinally elongate in lateral view, the cirrus sac thick-walled and muscular, and relatively shorter vitelline fields (13.6% of body length).

Lutztrema bhattacharyai (Pande, 1939) Travassos, 1944

HOSTS: **Copsychus saularis* (L.), magpie robin (Passeriformes: Muscicapidae: Turdinae); **Pycnonotus goiavier gourdini* Gray, yellow-vented bulbul (Passeriformes: Pycnonotidae).

HABITATS: Small intestine, liver, gall bladder.

LOCALITIES: Kasiqui, Petergas, Bukit Padang, Kapayan.

DATES: 27, 31 August, 6, 8, 18, 23, 26, 27 September 1960.

SPECIMENS DEPOSITED: No. 72725 (from *Copsychus*); No. 72726 (*Pycnonotus*).

DESCRIPTION (based on 101 adult worms, 11 measured; measurements are length by width by depth): Body 1,373–2,660 by 126–195 by 105–157; forebody 165–270 long, hindbody 1,065–2,215 long, forebody–hindbody length ratio 1:5.1–8.7; preoral space 2–12 long; oral sucker 52–87 by 58–80 by 47–57; acetabulum 104–190 by 119–175 by 83–121; sucker length ratio 1:1.77–2.18, width ratio 1:2.05–2.19, depth ratio 1:1.77–2.20; pharynx 22–41 by 25–39 by 22–29; cecum ending about halfway between ovary and posterior extremity; gonads preequatorial, in anterior third of hindbody; anterior testis 29–150 by 103–133 by 31–73, lying 58–160 postacetabular; posterior testis 48–143 by 106–136 by 30–63, lying 20–73 posterior to anterior testis; cirrus sac 60–98 by 41–46 by 26–28, overlapping acetabulum 15 to commencing 25–37 preacetabular; ovary 48–85 by 77–104 by 36–63; postovarian space 715–1,685 long; vitelline fields confluent anterodorsally, rarely ventrally more posteriorly; uterus usually crossing body between ovary and posterior testis, then crossing body in opposite direction between testes, occasionally ascending on same side of ovary and posterior testis before crossing body between testes; eggs operculate, 20 measuring 29–34 (32.1) by 16–22 (19.2).

Discussion

Our worms are smaller than that originally noted for this species from passeriform (Sturnidae) birds from India; however, ratios are similar. The original description did not indicate that the vitelline fields were confluent in part. Our collection contains one, four (in two), nine, 10, 19 and 36 worms from the small intestine of seven *C. saularis* and one, eight, and nine from the liver of three others; two worms are from the small intestine of one *P. goiavier* and four from the gall bladder of another.

Lyperosomum malaysiae sp. n. (Figs. 13–15)

HOST: *Pycnonotus zeylanicus* (Gmelin), yellow-crowned bulbul (Passeriformes: Pycnonotidae).

HABITATS: Small intestine, liver.

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LOCALITIES: Kasiqui, Tuaran.

DATES: 30 August, 1, 16 September 1960.

SPECIMENS DEPOSITED: No. 72727 (holotype); No. 72728 (paratypes).

DIAGNOSIS (based on one and three adult worms from the small intestine of two hosts and two from the liver of another, four measured): Body elongate, narrow, widest at acetabular level, 4,260–5,065 long by 410–455 wide by 490 deep. Forebody 410–610 long; hindbody 3,500–4,240 long; forebody–hindbody length ratio 1:6.7–8.5. Oral sucker ventral, longitudinally elongate, with posterior concavity, 165–198 by 167–177 by 121–126; preoral space 5–19 long. Acetabulum longitudinally elongate, 325–355 by 305–340 by 320–325. Sucker length ratio 1:1.76–1.97, width ratio 1:1.83–1.92, depth ratio 1:2.54–2.69. Prepharynx absent; pharynx pyriform, 73–78 by 87 by 85, anterior part within posterior concavity of oral sucker; esophagus 52–60 long; cecal bifurcation closer to oral sucker than acetabulum; ceca narrow, extent obscured by eggs. Excretory pore terminal.

Testes two, smooth, diagonal, all but lateral margins covered by eggs, transversely elongate, separated from each other and from acetabulum and ovary by uterine coils. Anterior testis sinistral, 110–145 long (in three) by 126 wide (in one), lying 105–165 postacetabular; posterior testis dextral, 98–115 long (in three) by 133 wide (in one), lying 335–340 postacetabular. Cirrus sac elongate, preacetabular, 98 long by 33 deep (in one), extending to cecal bifurcation or esophageal level. Seminal vesicle winding. Prostatic vesicle surrounded by few prostate cells. Cirrus muscular, protrusible. Genital pore just submedian, dextral.

Ovary dextral, smooth, transversely elongate, larger than testes, 110–190 long (in three) by 148–242 wide (in two), lying 265–320 posterior to posterior testis. Seminal receptacle postovarian, 107–160 by 125–145 by 116. Vitelline follicles small, in lateral fields, overlapping ceca, one field usually commencing at anterior testis and other at posterior testis level, terminating subequally, right field 1,285–1,815 long, left 1,610–1,700, longest field 34–36% of body length; postvitelline space 1,770–2,095 long, longer than longest vitelline field, distances 36–42% of body length. Uterus extensively coiled between acetabulum and posterior extremity, filling postvitelline space, with many coils between ovary and posterior testis, fewer between testes, undulating from acetabulum to genital pore. Metraterm thick-walled, shorter than cirrus sac, surrounded by gland cells. Eggs numerous, yellow-brown, operculate, 20 measuring 28–33 (30.4) by 17–22 (19.5).

Discussion

Ten species of this genus listed by Yamaguti (1971) resemble our new species. *L. anatis* Belogurov and Leonov, 1963, differs from our species in being smaller, and in having a larger cirrus sac and testes, the latter more nearly tandem, an ovary smaller than the testes, and a smaller sucker ratio. *L. charadrii* Belopolskaja, 1963, differs in having a smaller sucker ratio, an ovary smaller than the testes, and shorter vitelline fields (commencing between the posterior testis and ovary) relative to the body length, and the vitelline part of the body being much longer than the postvitelline part. *L. direptum* Nicoll, 1914, differs in being a larger species, in lacking an esophagus, and in having a smaller sucker ratio, the testes more nearly symmetrical, and the vitelline fields longer than the postvitelline space. *L. longicauda* (Rudolphi, 1809) Looss, 1899, differs in having a very short esophagus, a smaller forebody–hindbody length ratio, and a shorter postvitellarian space. *L. oswaldoi* (Travassos, 1920) Travassos, 1944, differs in having a wider body, larger testes (although the ovary is the same size as in our species), and vitelline fields longer than the postvitelline space. *L. petrovi* Kasimov in Skrjabin and Evranova, 1953, differs in having a larger sucker ratio, smaller eggs, and vitelline fields longer than the postvitelline space. *L. sarothrurae* Baer, 1959, differs in having a greater forebody–hindbody length ratio, a smaller sucker ratio, and gonads all about the same size, and the anterior testis overlapping the acetabular level. *L. schikhobalovi* Kasimov in Skrjabin and Evranova, 1953, differs in being larger with a relatively wider body, and in having a smaller forebody–hindbody length ratio, and vitelline fields longer than the postvitelline space. *L. skrjabini* (Solovev, 1911) Shtrom, 1940, differs in being larger, and in having a smaller forebody–hindbody length ratio, a relatively large cirrus sac, the vitelline fields longer than the postvitelline space, and larger eggs. *L. urocissae* Yamaguti, 1939,

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differs in having a forebody–hindbody length ratio of about 1:18, and the postvitelline space representing only about 25% of the total body length. *L. francolini* Oshmarin, 1970, also resembles our species, differing in having a much larger pharynx, much shorter vitelline fields, nearly subsymmetrical testes, and larger eggs, and the uterus not extending to the lateral body margins.

Proacetabulorchis dogieli Belopolskaja and Bykhovskaja-Pavlovskaja, 1953 (Fig. 16)

HOST: **Butorides striatus* (L.), little green or mangrove heron (Ciconiiformes: Ardeidae).

HABITATS: Liver, small intestine.

LOCALITIES: Bukit Padang, Petergas.

DATES: 9, 15 September 1960.

SPECIMENS DEPOSITED: No. 72729.

DESCRIPTION (based on 12 adult worms, five measured): Body elongate, slender, aspinose, 3,000–3,600 long by 425–560 wide. Forebody 720–885 long; hindbody 1,915–2,385 long; forebody–hindbody length ratio 1:2.3–2.8. Oral sucker subterminal ventral, longitudinally elongate, posteriorly 12–27 longer ventrally than dorsally, cuplike concavity posteriorly, thick muscle band just within posterior and posterolateral margins, 310–365 long ventrally, 286–338 long dorsally, 275–340 wide; thick postoral muscle band present; preoral body lacking. Acetabulum round to longitudinally elongate, 315–355 by 300–355. Sucker length ratio (using ventral oral sucker length) 1:0.94–1.09, width ratio 1:0.96–1.10. Pharynx usually transversely elongate, 68–87 by 78–94, anterior part within posterior concavity of oral sucker; esophagus 92 long in two contracted worms, 126–150 long in three relaxed worms; cecal bifurcation closer to oral sucker than acetabulum, lying 115–325 anterior to latter; ceca narrow, conspicuously cell-lined, terminating 440–625 from posterior extremity, latter distances 14.7–17.8% of body length.

Testes two, subsymmetrical in nine worms, symmetrical in one, slightly lobed, usually longitudinally elongate. When diagonal: anterior testis dextral in four or sinistral in five, entirely preacetabular in four to overlapping acetabulum in five; posterior testis always overlapping acetabulum. When symmetrical: both testes overlapping acetabulum. Right testis 138–206 by 75–157, left 97–194 by 97–148. Vasa efferentia uniting to form short vas deferens. Cirrus sac elongate, thick-walled, muscular, widest at about midlength, 225–305 by 85–100, commencing 50–145 preacetabular on same side as posterior testis if testes subsymmetrical or medianly if testes symmetrical, extending to level of posterior half of esophagus. Seminal vesicle saccular, 133–213 by 75–90, filling posterior 59–76% of cirrus sac length. Pars prostatica small, lying entirely anterior to seminal vesicle to being completely overlapped by latter, surrounded by few prostate cells. Cirrus muscular, winding, protrusible. Genital pore right or left of esophagus, occasionally nearly median and ventral to latter.

Ovary slightly lobed, smaller than testes, usually submedian, occasionally median, 62–90 by 88–116, lying 355–550 postacetabular. Seminal receptacle 102–130 by 70–92, postovarian, contiguous with or separated from latter, submedian, rarely median. Mehlis' gland large, well developed, submedian on side opposite seminal receptacle. Laurer's canal muscular, on same side as Mehlis' gland, intercecal, extending laterally or posterolaterally to dorsal surface. Vitellaria in lateral extracecal fields from short distance postacetabular to 915–1,180 from posterior extremity, latter distances 30.5–33.7% of body length. Uterus mainly intercecal, winding, descending to near posterior extremity, ascending dextral or sinistral to posterior part of cirrus sac. Metraterm thick-walled, muscular, commencing near anterior part of cirrus sac, dorsal to latter, surrounded by gland cells. Eggs numerous, brown, operculate, 15 measuring 26–36 (31.7) by 16–22 (18.5).

Excretory bladder long, tubular, commencing 295–395 postovarian, surrounded by gland cells just before terminal pore; collecting ducts reaching pharyngeal level, entering bladder just posterior to anteriormost tip.

Discussion

P. dogieli is found in charadriiform (Scolopacidae) birds from western Siberia to the Komander Islands lying off the Kamchatka Peninsula. Bykhovskaja-Pavlovskaja (1962) transferred this species from *Proacetabulorchis* Gogate, 1940, to *Platynotrema* Nicoll, 1914; Odening (1964) concurred. Yamaguti (1958, 1971) retained it in its original genus. We

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concur with Yamaguti as the body shape and distribution of the vitellaria are characteristic of *Proacetabulorchis* rather than *Platynotrema*. *P. dogieli* was placed in the latter genus primarily because of having symmetrical testes, whereas the testes in the former genus are supposed to be tandem. However, in *P. dogieli*, while the testes are frequently symmetrical, they are somewhat diagonal in the original illustration and in most of our worms. While the testes are essentially tandem in *Proacetabulorchis prashadi* Gogate, 1940, the original illustration shows them somewhat diagonal; this is their position in the five specimens of *P. prashadi* reported in this paper. Therefore, the relationship of the testes to one another is variable in these two species.

Our collection contains two adult worms from the small intestine of one host and 10 from the liver of another. Our worms basically fit *P. dogieli*; no mention is made of the shape and structure of the oral sucker, but this may have been overlooked.

Proacetabulorchis prashadi Gogate, 1940

HOST: **Halcyon chloris* (Boddaert), white-collared kingfisher (Coraciiformes: Alcedinidae).

HABITAT: Liver.

LOCALITIES: Tuaran, Petergas.

DATES: 9, 15 September 1960.

SPECIMENS DEPOSITED: No. 72730.

Discussion

Our collection contains one and four adult worms from two hosts. This species has been reported from the same host genus from Burma, Vietnam, and Japan. The testes, while being nearly tandem, are distinctly diagonal.

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In Memoriam

Clark P. Read

February 4, 1921–December 24, 1973

Member since 1954

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Sarcocystis fusiformis: Development of Cysts in Calves Infected with Sporocysts from Dogs

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ABSTRACT: Fourteen calves were orally inoculated with sporocysts excreted by beagle puppies that had ingested raw beef infected with *Sarcocystis fusiformis*. Eight control calves were uninoculated. No parasites were found in histological specimens obtained from calves 1 to 19 days after inoculation. Schizonts were observed in the mesenteric lymph nodes of a calf 20 days after inoculation. Between 26 and 33 days, nine calves became severely ill and died or were near death and were killed. Schizonts were found in the following organs of these animals: adrenal glands, cecum, cerebellum, cerebrum, diaphragm, esophagus, eye, auricle and ventricle of the heart, ileum, jejunum, kidney, liver, lung, mesenteric lymph nodes, pancreas, spleen, skeletal muscle, testicle, thyroid, tongue, and urinary bladder. Mononuclear basophilic organisms resembling metrocytes were first observed in diaphragm, esophagus, heart, and kidney 26 days after inoculation. Cysts containing zoites and metrocytes were first observed in the ventricle of a calf at 33 days. Between 40 and 54 days, cysts containing zoites and metrocytes were found in the diaphragm, esophagus, heart, skeletal muscles, and tongue; no schizonts were observed at this time. No illness was observed and no parasites were found in the organs of any control animal examined.

Sporulated sporocysts containing four sporozoites occur in the feces of dogs fed *Sarcocystis*-infected beef (Heydorn and Rommel, 1972; Mahrt, 1973; Fayer and Leek, 1973). When calves were inoculated orally with such sporocysts, schizonts were found in many of the internal organs (Fayer and Johnson, 1973). The present report describes the development of the cyst stage in bovine muscle and muscular organs, thus completing the description of the life cycle of *Sarcocystis fusiformis*.

Materials and Methods

Twenty-two 8- to 45-week-old Holstein–Friesian calves were raised at the Animal Parasitology Institute in the absence of carnivores. In eight separate experiments, 14 calves were fed their normal daily grain ration mixed with an aqueous suspension of 220,000 to 1 million *S. fusiformis* sporocysts obtained as previously described (Fayer and Leek, loc. cit.) (Table 1). Eight control calves were given grain without sporocysts. Infected calves that died, or became recumbent and were killed, were examined postmortem and the following tissues taken for histological examination: adrenal glands, cecum, cerebellum, cerebrum, diaphragm, esophagus, eye, auricle and ventricle of the heart, ileum, jejunum, kidney, liver, lung, mesenteric lymph node, pancreas, spleen, skeletal muscle, testicle, thyroid, tongue, and urinary bladder. In addition, 1 and 2 days after inoculation, biopsy tissue was taken from the small intestine of calf #14 at both 2 and 4 ft distal to the pylorus, and on day 3 the calf was killed and tissues taken for examination. Biopsy material from both the kidney and the mesenteric lymph nodes was obtained on day 2 and again on day 9 from calf #16 and on days 3 and 10 from calf #15. These calves were then killed on days 19 and 20, respectively, at which time tissues were taken. Four of the eight control animals also were killed, a postmortem examination made, and tissues taken.

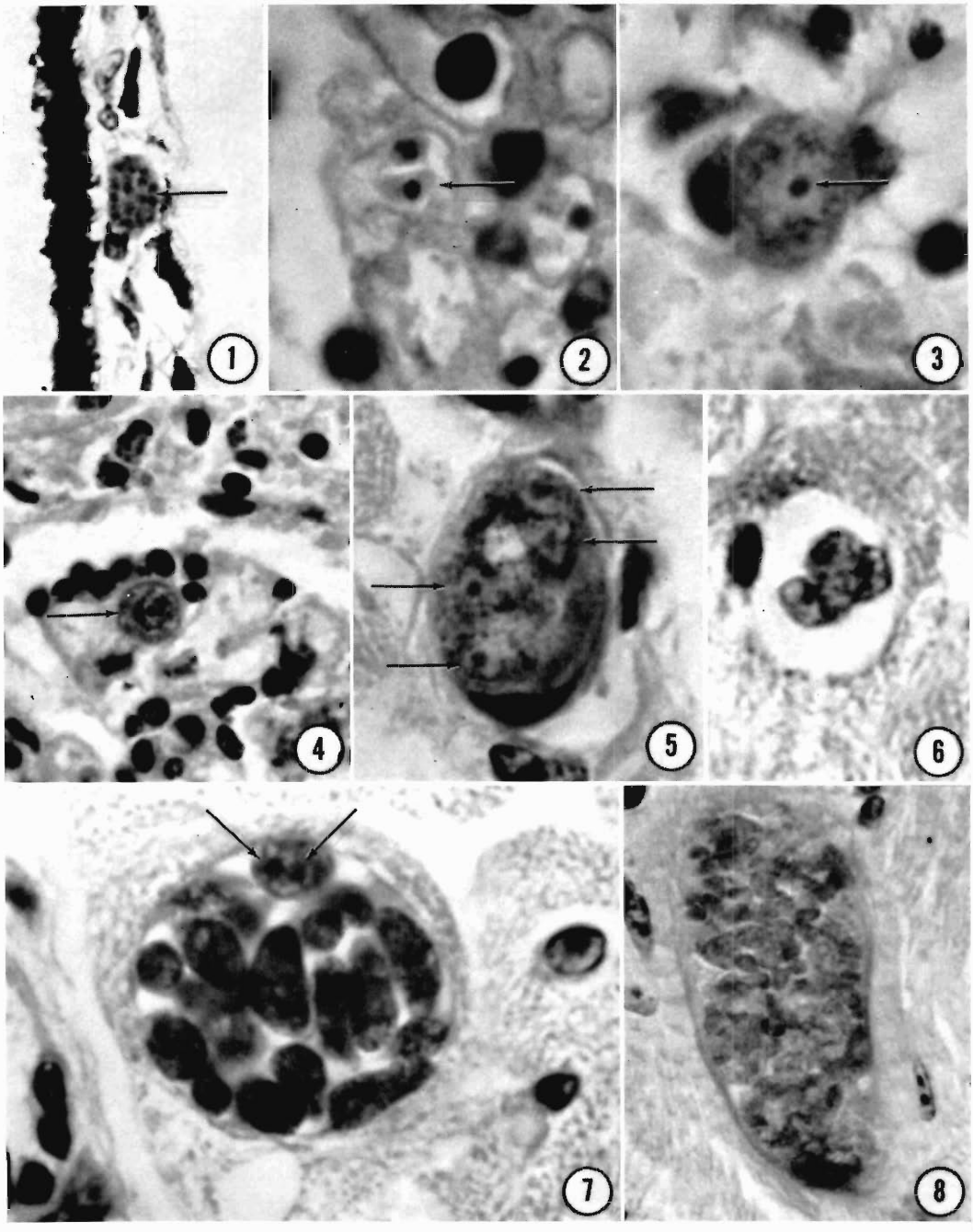
Tissues were fixed in either neutral buffered 10% formalin or Technicon FU-48.* They were embedded in paraffin, sectioned at 5 to 7 μ, and stained with hematoxylin and eosin or Heidenhain's iron hematoxylin.

Results and Discussion

No parasites were observed in the biopsy specimens from the small intestine, in kidney and mesenteric lymph node biopsies obtained

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Figures 1–8. Photomicrographs of hematoxylin–eosin-stained tissue sections containing developmental stages of *Sarcocystis fusiformis*. 1. Schizont (arrow) in choroid of the eye, 32 days. 640×. 2. Merozoite (arrow) in glomerulus of kidney, 33 days. 1,600×. 3. Mononuclear basophilic organism resembling a metro-

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Table 1. Dosage levels of calves inoculated with *Sarcocystis fusiformis* sporocysts.

Exp. no.	Calf no.	No. of sporocysts inoculated	Day of death
1	1	1×10^6	27k
	2	1×10^6	40k
	3	Control	27k
	4	Control	40k
2	5	$1 \times 10^{6*}$	54k
	6	$1 \times 10^{6*}$	27d
3	7	4.5×10^5	26k
	8	4.5×10^5	33d
	9	Control	—
	10	Control	—
4	11	2.6×10^5	33k
	12	Control	35k
5	13	2.7×10^5	46k
6	14	4.4×10^5	1 biopsy intestine 2 biopsy intestine 3k
7	15	3.2×10^5	3 biopsy kidney, lymph node 10 biopsy kidney, lymph node 20k
	16	3.2×10^5	2 biopsy kidney, lymph node 9 biopsy kidney, lymph node 19k
8	17	2.2×10^5	31k
	18	2.2×10^5	32d
	19	2.2×10^5	27k
	20	Control	34k
	21	Control	—
	22	Control	—

k = Day after first inoculation on which calf was killed.
d = Day after first inoculation on which calf died.
* Five daily inoculations of 200,000 sporocysts.

on days 2, 3, 9, and 10, in any tissues from days 3 and 19, or in any of the control animals (Table 1).

Immature schizonts were first observed in the mesenteric lymph nodes of calf #15, 20 days after inoculation. Between 26 and 54 days after inoculation all infected calves became severely ill and died or were recumbent and were killed. In tissues taken from calves 26 to 33 days after inoculation immature schizonts were found in the choroid plexus of the brain, in the ciliary body and the nonpigmented portion of the choroid of the eye (Fig. 1), in the lamina propria and muscularis mucosae of the jejunum and ileum, in the tunica of the testicle, in the thyroid, and in those organs previously reported by Fayer and Johnson (1973) which include the adrenal glands, cecum, cerebellum, cerebrum, diaphragm, esophagus, auricle and ventricle of the heart, kidney, liver, mesenteric lymph nodes, pancreas, spleen, skeletal muscle, tongue, and urinary bladder. Mature schizonts containing merozoites were observed in the adrenals, lymph nodes, jejunum, and kidney. The glomeruli of the kidneys were the most heavily parasitized tissues, and contained most of the mature schizonts that were observed. In addition, this was the only location where individuals or pairs of merozoites were observed outside the schizonts (Fig. 2). These merozoites were small, averaging 5.4 by 2.1 μ ($N = 10$), with a pale eosinophilic cytoplasm and a distinct basophilic nucleus. One end appeared rounded and the other pointed.

At 26 days after inoculation, mononuclear basophilic organisms which appeared quite similar to the metrocytes of *Sarcocystis* described by Senaud (1967) and Melhorn and Scholtyseck (1973), were observed in the diaphragm, esophagus, heart, and kidney of calf #7. Organisms identical to these were found in the glomeruli of the kidney of calf #6 at 27 days and in the heart (Fig. 3), tongue, lung, and glomeruli (Fig. 4) of calf #8 at 33 days. A cyst containing four metrocytes (Fig. 5) was also found in the musculature of the tongue of this calf and typical cysts of *Sarcocystis*, containing as many as 50 zoites and metrocytes, were also observed at 33 days in the ventricle of calf #11. In calves #2, #5, and #13 killed 40, 54, and 46 days after inoculation, respectively, no schizonts were observed in any of the tissues examined nor were mononuclear basophilic organisms or cyst stages found in any tissues other than the diaphragm, esophagus, heart, skeletal muscle, and tongue. The fate of the mononuclear basophilic organisms previously observed in the lungs and

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cyte in the ventricular myocardium, 33 days. Organism has a large nucleus and distinct nucleolus (arrow). 1,575×. 4. Mononuclear organism (arrow) in the glomerulus of the kidney. 640×. 5. Young cyst stage with four metrocytes (arrows) in the musculature of the tongue, 33 days. 1,575×. 6. Three-metrocyte stage surrounded by a vacuole in the myocardium, 54 days. 1,575×. 7. Young cyst with zoites in center and metrocytes at periphery, in heart, 54 days. Metrocyte containing two developing organisms (arrows). 1,575×. 8. Cyst in the muscular portion of the tongue, 54 days. 640×.

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kidney is not known. They may precede another schizogonic generation or be metrocytes which fail to develop in locations other than muscles.

At 54 days after inoculation mononuclear basophilic organisms as well as young cyst stages with three to 15 metrocytes and large cysts with many zoites and metrocytes were observed. Some young stages did not appear to be surrounded by a host cell or a cyst wall but appeared to be surrounded by a large vacuole (Fig. 6). Larger stages were observed in which well-developed zoites were contained in the central portion and metrocytes were located at the periphery (Fig. 7). Such cysts were surrounded by a distinct wall, and this in turn was surrounded by muscle fiber. Organisms within these and other still larger cysts were quite basophilic and the surrounding wall eosinophilic (Fig. 8). Zoites in these cysts averaged 8.3 by 3.1 μ ($N = 15$) with a range of 6.2 to 11.6 by 2.3 to 3.9 μ. The cysts themselves varied greatly in shape from round to slender and elongate. The largest cyst observed was in the ventricular myocardium and measured 39 by 101.6 μ.

The presence of these cyst stages in calves inoculated with sporocysts obtained from dogs and the absence of such cysts in uninoculated control animals indicates that these sporocysts are a source of *Sarcocystis* for cattle. Since dogs became infected by eating raw beef infected with S. *fusiformis* it appears that the life cycle of S. *fusiformis* can be completed in two vertebrate hosts in somewhat the same manner as *Toxoplasma gondii*, in which a cat–mouse–cat cycle occurs (Hutchison et al., 1970; Sheffield and Melton, 1970; Frenkel et al., 1970; Overdulve, 1970) and as in *Sarcocystis* species presumed to be S. *muris* in which a cat–mouse–cat cycle also occurs (Wallace, 1973).

Postmortem and histopathological findings will be reported in detail elsewhere.

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Research Note

Diplotriaena obtusa (Rud., 1802) and *Plagiorchis maculosus* (Rud., 1802) Collected from a Purple Martin, *Progne subis*, in Canada

Numerous specimens (21 males, 18 females) of *Diplotriaena obtusa* (Rud., 1802) were removed from the body cavity of a purple martin, *Progne subis*, found dead in Ottawa, Ontario. The only previous report of this species in North America is that of Anderson (1961, Can. J. Zool. 39: 377) who found a single female in a bank swallow, *Riparia riparia*, from Ontario.

In redescribing *D. obtusa*, Anderson (1959, Parassitologia 1: 195–307) noted that the characters separating it from the morphologically similar species, *D. tricuspis* (Fedtschenko, 1874), are the structure of the tridents and of the right spicule, and that these characters require reexamination as additional specimens of *D. obtusa* become available.

The morphology of *D. obtusa* recovered from the purple martin is similar to that of the specimens described by Anderson (1959, loc. cit.). The tridents range in length from 135–180 (avg 158) μ. The tip of the trident is rounded, quite distinct from that of *D. tricuspis* which is flat or concave. The spicules of *D. obtusa* are 540–775 (avg 625) μ and 1,050–1,320 (avg 1,165) μ in length. These are somewhat longer than described by Anderson (1959, loc. cit.) but fall within the range of those described by Sonin (1962, Trudy gel'mint. Lab. 12: 139–165). Comparison with *D. tricuspis* shows that the right spicule of *D. obtusa* has much sharper twists and that the distal twist is much closer to the end.

Specimens of *Plagiorchis maculosus* (Rud., 1802) were recovered from the intestine of this same bird. The morphology is in agreement with that of specimens described by Angel (1959, Trans. Roy. Soc. S. Aust. 82: 265–281). This species has been reported only once previously from North America and was found in the American herring gull (Threlfall, 1968, Can. J. Zool. 46: 1119–1126). Rayner (1932, Sci. Agr. 12: 307–309) reported finding an unidentified species of *Plagiorchis* from a purple martin caught near Montreal, Quebec.

Representative specimens have been deposited in the USNM Helminthological Collection Nos. 72806 *Diplotriaena obtusa* and 72805 *Plagiorchis maculosus*.

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Research Note

Management to Control Helminth Parasitism: Infectiousness of Pastures that Have Been Rested or Grazed by Resistant Cattle

The practices of resting pastures or using resistant animals as "vacuum cleaners" have been recommended as methods for reducing pasture populations of free-living stages of parasitic nematodes. A small experiment was conducted to examine these practices.

Equal amounts of thoroughly mixed bovine manure, containing nematode eggs, were placed on six pasture plots between 22 May and 1 June. The plots, each 0.006 hectare, had been planted to Kentucky bluegrass and clover and mowed before they were contaminated.

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Table 1. Worm burden of calves that grazed plots, infectious with nematode larvae, with or without prior grazing by resistant cattle.

Period since end of contamination	3 weeks		8.6 weeks			
Prior handling of plot	Ungrazed		Grazed		Ungrazed	
Calf No.	1	2	3	4	5	6
Haemonchus contortus	3,375	1,874	200	250	250	50
Ostertagia ostertagi	513	500	1,498	1,498	6,370	1,235
Trichostrongylus axei	788	1,650	3,630	2,053	17,000	2,240
Cooperia oncophora	3,300	1,238	7,313	4,750	17,175	2,813
Cooperia punctata	3,300	1,350	719	438	750	125
Oesophagostomum radiatum	6,534	2,867	3,297	1,152	2,871	847

The test calves were helminth-free and 3 to 5 months old.

To estimate the extent of larval migration onto the herbage at 3 weeks, calves 1 and 2 were put on two of the plots on 23 June, and kept there until they had grazed them off, in 13 and 16 days, respectively. Simultaneously, two resistant 3-year-old cattle not passing nematode eggs were put on two more plots, which they grazed off in 4 and 5 days, respectively.

Eight and six-tenths weeks after the end of contamination, calves 3 and 4 were placed on the two plots previously grazed by the resistant cattle; calves 5 and 6 were placed on previously ungrazed plots. Calf 6 had consumed all the forage on 95% of its plot, but some remained on 5% when the calf had to be removed to prevent recontamination of the plot.

After the calves had completed grazing, they were kept in a barn for 3 weeks and then necropsied to determine the numbers of worms present (Table 1). The numbers of *Haemonchus contortus* and *Cooperia punctata* recovered from calves that grazed plots 8.6 weeks after contamination were considerably fewer than those recovered from calves that grazed plots 3 weeks after contamination. A possible explanation of this finding is that conditions for development and migration of these species onto the herbage had been good, and that many of their larvae on the herbage had died in that interval. Conversely, more *Ostertagia ostertagi* and *Trichostrongylus axei* were recovered from calves grazed 8.6 weeks after contamination of the plats than at 3 weeks; this finding indicated that optimal conditions for migration of these species onto the forage occurred later than conditions did for *H. contortus* and *C. punctata*, and that *O. ostertagi* and *T. axei* survived longer. The infectiousness of the forage with *Cooperia oncophora* and *Oesophagostomum radiatum* appeared to be about the same at 3 weeks as at 8.6 weeks.

The procedures in this study were not adequate to control *O. ostertagi*. This finding might have been expected from previous studies of development, migration onto the herbage, and survival of larvae in this area (Goldberg, 1968, J. Parasit. 54: 856–862). In those studies, on the average, peak infectiousness of the herbage occurred at about 6 weeks after deposition of feces in May. The present study indicated that, in general, grazing off plots by resistant animals at 3 weeks after the end of contamination did not appreciably reduce their infectiousness for calves that regrazed the plots at 8.6 weeks. Also, after resting plots for 8.6 weeks, an appreciable percentage of *T. axei*, *C. oncophora*, and *O. radiatum* larvae were still capable of infecting calves. However, there was a considerable reduction in the percentage of *H. contortus* and *C. punctata* between 3 and 8.6 weeks.

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Research Note

Anticoccidial Activity of the Megalomicin Complex

Macrolide antibiotics produced as *Streptomyces* fermentation products, particularly spiramicin, have been reported to possess anticoccidial activity (Ball, 1959, J. Comp. Path. 69: 327–333; Ball and Warren, 1966, J. Comp. Path. 76: 255–259; Cuckler and Malanga, 1957, Antibiotics Annual 1956–1957: 592–595). This paper reports the anticoccidial activity of the megalomicin complex, a novel *Micromonospora*-produced antibiotic complex (Weinstein et al., 1969, J. Antibiotics 22: 253–258). The effect of structural changes of components of the megalomicin complex on anticoccidial activity is also reported.

Efficacy determinations were conducted by administering feed containing selected concentrations of the antibiotic to groups of four to 10 11-day-old Cobb white broiler males. Chicks were wingbanded, distributed by weight (Gardiner and Wehr, 1950, Proc. Helm. Soc. Wash. 17: 25–26), and placed on medicated feed 48 hr prior to infection; continued throughout the experimental period. A vitamin K- and antibiotic-free feed was used for these studies. Chicks were infected with an estimated 10^5 sporulated oocysts of *E. tenella*. Eight days after infection, all surviving birds were autopsied. Weight changes, fecal dropping scores (0 = normal to ++++ = severe), cecal lesions (0 = no gross pathology to 3 = enlarged ceca, cecal core present, 4 = death due to coccidiosis), estimates of numbers of oocysts in the ceca at autopsy, and mortality data were recorded.

Efficacy trials indicated that 3′4′ diacetyl megalomicin A (megalomicin C of Weinstein et al., 1969, loc. cit.) was effective at 0.025% (Table 1). Correlation of efficacy with mortality results in a decreasing order of efficacy as follows: 3′4′ diacetylmegalomicin A<3′4′ diacetyl megalomicin A tartarate<megalomicin A<4′2″4‴ triacetylmegalomicin A = 3′4′ diacetyl 2″ propionyl megalomicin A<4′2″4‴ tripropionyl megalomicin A. A similar correlation may be drawn between cecal lesion scores and fecal dropping scores; however, the differences are not as striking. At 0.025% and higher concentrations of the 3′4′ diacetyl megalomicin A the cecal oocyst estimates were markedly reduced.

Attempts to correlate structure with anticoccidial activity of macrolide antibiotics erythromicin, tylosin, spiramicin, rosamicin, and the megalomicins using available published data (Merck Index, 8th ed.) indicates that no apparent correlation exists between aglycone

Table 1. Anticoccidial activity of various megalomicin components and derivatives and erythromicin in vitamin K-deficient antibiotic free feed.

Compound	Per cent diet	Mortality died/total	Average cecal lesion per bird	Relative fecal score[2]	Average cecal oocyst content ($\times 10^6$) per bird[1]
3′4′ diacetyl Megalomicin A	0.050	0/12	1.33	—	0.02
	0.025	0/16	1.56	—	0.83
3′4′ diacetyl Megalomicin A tartrate	0.025	0/12	1.50	+	4.16
3′4′ diacetyl 2″ propionyl Megalomicin A	0.025	2/12	1.67	+++	6.74
4′2″4‴ tripropionyl Megalomicin A	0.025	4/12	2.00	++++	4.66
4′2″4‴ triacetyl Megalomicin A	0.025	2/12	1.75	+++	4.11
Megalomicin A phosphate	0.025	0/12	1.67	+++	6.52
Inoculated untreated control	- -	0/12	0.33	—	0.10
Infected untreated control	—	4/12	2.50	++++	2.31
Erythromicin	0.025	4/12	2.67	++++	7.85

[1] Based on surviving birds.
[2] — = None, + = Modest, ++ = Medium, +++ = Intense, ++++ = Extremely intense.

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ring size, molecular weight, numbers, types, and locations of sugars on the aglycone ring, and positions of minor substituents on the aglycone ring. *Streptomyces*-produced macrolide antibiotics with anticoccidial activity have a mycarose–mycaminose sugar in the 5 position of the aglycone ring. Components of the megalomicin complex have a desosamine sugar in the 5 position and a mycarose sugar in the 3 position of the aglycone ring.

Natural acetylation of the mycarose sugar (3′4′ diacetyl megalomicin A) produced a marked increase in anticoccidial activity. Whether this activity is due to the 3′ or 4′ acetylation alone or the 3′4′ diacetyl combination is yet to be determined. Substitution of a propionyl group on the 2″ position of the megalomicin desosamine sugar apparently antagonizes the anticoccidial activity of the 3′4′ diacetyl substituents of the mycarose sugar. Cecal oocyst counts indicate the phosphate or tartrate salts adversely influence the anticoccidial activity of their respective bases when administered in feed.

Modification of substituent sugars of the megalomicin complex components markedly influences anticoccidial activity of this antibiotic complex. Additional studies are necessary to further define the structure–anticoccidial relationships of the megalomicins. This paper represents the first published report of antiparasitic activity of *Micromonospora*-produced antibiotics.

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Research Note

Passage through Rouen Ducks of the Metacercariae of *Fasciola hepatica*[1]

According to Samson and Wilson (1973, Proc. Helm. Soc. Wash. 40: 292–293), Rouen ducks used as potential biological control agents substantially reduced natural populations of one of the snail vectors of the common liver fluke, *Fasciola hepatica*, on small areas of boggy pasture. In addition to reducing the snail population, ducks feeding on snails and aquatic vegetation as control agents within an enzootic fluke area would undoubtedly also ingest some fluke metacercariae. To determine if passage of metacercariae through the duck's digestive tract would destroy the metacercariae or reduce their infectivity for sheep, two experiments were carried out.

In a preliminary test, 200 metacercariae obtained from laboratory-infected *Lymnaea* (*Stagnicola*) *palustris* were fed to a 9-week-old pen-raised Rouen duck and the fecal material was collected for 48 hr, a period felt to be adequate for complete evacuation of the alimentary tract as reported by poultry specialists. The feces was then mixed with water and washed through a 40-mesh screen to remove large pieces of undigested feed. The material which passed through the 40-mesh screen was then washed in a 200-mesh screen to remove suspended material. All fecal material retained on the 200-mesh screen was washed into a beaker, settled, centrifuged at

Table 1. Development of metacercariae in lambs after passage through ducks; 200 metacercariae per duck.

No. of lambs	Dose given	No. of flukes recovered per lamb: After 84 days	After 97 days	Avg	Reduction
On test—6	Duck fecal material	0 0 1 2	0 1	0.67	99%
Control—3	200 metacercariae	80 92	10	61.0	

[1] This work was carried out in cooperation with the New Mexico State University Agricultural Experiment Station and is reported as Journal article 471 of that Station. Las Cruces, New Mexico 88003.

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800 rpm (approximately 100 *g*), and given orally to a clean lamb by drench gun. Control tests showed that metacercariae were apparently not damaged by this screening and centrifuging procedure. At the same time 200 metacercariae from the same lot were centrifuged and given orally to a clean control lamb. On day 65 of the test, the duck and both lambs were killed and examined for flukes. No flukes were recovered from the duck nor from the lamb given the fecal material, whereas 67 flukes were recovered from the liver of the lamb given metacercariae directly.

To confirm the results of the preliminary test, six ducks were given 200 metacercariae each, the fecal material was collected individually for 72 hr, processed as before, and given to six test lambs. Three control lambs received 200 metacercariae directly in water. Four of the six test lambs and two controls were killed 84 days after dosing, and two test lambs and one control were killed 97 days after dosing. Results of these tests are shown in Table 1.

No flukes were found in three of the test lambs, but one or two flukes were found in each of the other three. Apparently there was a 99% reduction in the number of viable metacercariae after passage through the ducks. The ducks were also examined, but no evidence of infection was found. Ducks, therefore, might reduce the incidence of fascioliasis by ingesting metacercariae as they feed on the snail vectors and vegetation within small enzootic areas such as are found in boggy pastures, irrigation ditches, springs, and seeps.

We wish to thank Dr. Joseph P. E. Morrison, Associate Curator, Division of Mollusks, National Museum of Natural History, Washington, D. C., and Dr. Richard H. Russell, Department of Biological Sciences, University of Arizona, Tucson, for their help in identifying the snails.

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Research Note

Parasitic Helminths of Bats from the Southwestern United States and Mexico

During the summer of 1969, representatives of 17 species of bats were collected in the southwestern United States, western Mexico, and the Panama Canal Zone. Following their employment in physiological studies (Studier and Wilson, 1970, Comp. Bioch. Physiol., 34: 251–262; Studier, 1970, Comp. Bioch. Physiol. 35: 935–943) the bats were examined for endoparasites, and blood smears were taken for detection of blood parasites. Findings of these examinations are summarized in Table 1.

Blood parasites were uniformly absent in bats listed in Table 1 and in the following species: 8 *Pteronotus suapurensis*, 10 *Myotis nigricans*, 1 *Micronycteris hirsuta*, 10 *Molossus coibensis*, 1 *Trachops cirrhosus*, 3 *Saccopteryx bilineata*, 1 *Uroderma bilobatum*, 7 *Vampyrodes carracioli*, 10 *Artibeus jamaicensis*, 6 *A. cinereus*, 1 *A. lituratus*, 3 *Carollia castanea*, 3 *C. perspicillata*, 4 *Desmodus rotundus*, and 1 *Lonchophylla robusta*, all from the Panama Canal Zone; 15 *Plecotus townsendi* from New Mexico; and 1 *Myotis evotis* from California. Although blood parasites have been previously reported from several of the above species, Marinkelle (1966, Trans. Roy. Soc. Trop. Med. Hyg. 60: 109–116) has noted that hemoflagellates, at least, are rarely detected in bats without concentration, xenodiagnostic, or culture methods.

Five species of helminths were encountered (Table 1), and three new host records are now established. *Plagiorchis micracanthos* is widely distributed in North America and occurs in several species of bats (Macy, 1931, J. Parasitol. 18: 28–33; Williams, 1962, Ohio J.

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Table 1. Parasitic helminths of bats from the southwestern United States, and Mexico.

Host	Location	Parasite	No. examined	No. infected
Vespertilionidae				
Plecotus phyllotis	Nevada	—	4	0
Eptesicus fuscus	New Mexico	—	10	0
Antrozous pallidus	Nevada	—	1	0
Lasiurus cinereus	Arizona	*Cycloskrjabinia taborensis* (Loewen, 1934) Spassky, 1951 (Cestoda)	9	2
Pipistrellus hesperus	Nevada	*Plagiorchis micracanthos* Macy, 1931 (Trematoda)	10	1
Myotis lucifugus	New Mexico	*P. micracanthos*	13	3
		Rictularia lucifugus Douvres, 1956 (Nematoda)	13	1
M. thysanodes	New Mexico	*Vampirolepis gertschi* (Macy, 1947) Spassky, 1954 (Cestoda)	40	2
M. volans	Nevada	—	2	0
M. yumaensis	Arizona	—	10	0
Molossidae				
Eumops perotis	California	—	3	0
Molossus ater	Sinaloa, Mexico	*Molinostrongylus delicatus* (Schwartz, 1927) Travassos, 1937 (Nematoda)	12	2
Phyllostomatidae				
Glossophaga soricina	Sonora, Mexico	—	10	0
Leptonycteris sanborni	Sonora, Mexico	—	10	0
Pteronotus davyi	Sonora, Mexico	—	10	0
P. parnelli	Sonora, Mexico	—	4	0
P. psilotus	Sonora, Mexico	—	6	0
Natalidae				
Natalus stramineus	Sonora, Mexico	—	10	0
Emballonuridae				
Balantiopteryx plicata	Sonora, Mexico	—	10	0

Sci. 62: 273; Ubelaker, 1966, Am. Midl. Nat. 75: 199–204; Nickel and Hansen, 1967, Am. Midl. Nat. 78: 481–486; Cain and Studier, 1972, Southwest. Nat. 17: 104–105), but this is the first report of this species from *Pipistrellus hesperus*. *Cycloskrjabinia taborensis*, a cestode apparently restricted to bats of the genus *Lasiurus*, has previously been reported only from the Midwest (Loewen, 1934, Trans. Kans. Acad. Sci. 37: 237–261; Stunkard, 1961, J. Parasitol. 47: 847–856; Blankespoor and Ulmer, 1970, Proc. Iowa Acad. Sci. 77: 200–206). This report extends the parasite's range to the southwestern United States.

The nematode *Molinostrongylus delicatus* is commonly encountered in southwestern bats (Jameson, 1959, Southwest. Nat. 4: 61–65; Cain, 1966, J. Parasitol. 52: 351–357), but this is the first report of this species from *Molossus ater*. The occurrence of *Rictularia lucifugus* in *Myotis lucifugus* from New Mexico represents an extension of that nematode's range to the southwestern United States.

The most significant aspect of this study is the occurrence of *Vampirolepis gertschi* in *Myotis thysanodes*, the fringe-tailed bat. *V. gertschi* has been recorded from *Tadarida brasiliensis* (Cain, 1966, loc. cit.), but the present study is the first report of it or any endoparasite from *M. thysanodes*. Although this host is not commonly dealt with in surveys for parasites, we have examined 40 individuals over a 10-year period, only two of which yielded parasitic helminths. Thus, *M. thysanodes*, unlike most insectivorous bats, appears to be relatively free of endoparasites.

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Research Note

Larval Nematode, *Contracaecum* sp., in the Hydromedusa, *Polyorchis penicillatus* (Eschscholtz)

Because of current interest in the life histories of heterocheiloid nematodes of food fish and in the use of parasites of marine plankton as indicators of their definitive hosts (Noble, 1972, Trans. Am. Microscop. Soc. 91: 90–91), the following observations are given.

Three larval *Contracaecum* sp. are reported for the first time from the mesoglea near the radial canals of two specimens of a small planktonic hydromedusa, *Polyorchis penicillatus* (Eschscholtz), which were collected from San Francisco Bay in October 1912 and January 1913. The nematodes were sent to me for identification by Ronald J. Larson, Division of Echinoderms, Smithsonian Institution, Washington, D. C. They have been deposited in the National Parasite Collection as USNM Helminthological Collection No. 72479.

Contracaecum spp. are parasites of the upper digestive tract of piscivorous fishes, birds, and mammals. Larval *Contracaecum* occur in nearly all marine fish examined except elasmobranchs. According to Berland (1961, Sarsia 2: 1–50), it is uncertain whether one or two hosts are commonly involved in the life cycle prior to infection of the definitive host. If two hosts are involved, planktonic invertebrates or, occasionally, fish may serve as transfer hosts in which growth but no molt occurs. Larger fish may serve as intermediate hosts in which the second molt occurs. The final two molts occur in the definitive host (Berland, loc. cit.).

Huizinga (1966, J. Elisha Mitchell Sci. Soc. 82: 181–195) and Kahl (1936, Ztschr. Parasitenk. 8: 509–520) have reviewed the invertebrates that have been reported as hosts for *Contracaecum* spp. These include various copepods, shrimp, mysids, arrowworms, cephalopods, and medusae. In addition, a sea-anemone (Schuurmans Stekhoven and Allgen, 1941, Zool. Anz., Leipzig 136: 76–77), polychaetes (Margolis, 1971, J. Fish. Res. Bd. Canada 28: 1385–1392), and a ctenophore (Boyle, 1966, Trans. Roy. Soc. New Zeal., Zool. 8: 51–62) are known hosts for members of this genus. All known references to "medusae" serving as hosts of *Contracaecum* spp. appear to be derived from Wülker (1929, Zool. Anz., Suppl. 4: 147–157), who did not list specific medusae.

The larvae reported herein are 3.86–5.93 mm long, with esophagus 583–791 μ long, nerve ring 85–230 μ from anterior end, intestinal cecum 280–426 μ long. Two specimens have tails with a sharp terminal spine and the spiny "cactus" tail of the next stage visible within the cuticle. They also have a boring tooth and are similar to third-stage larvae of *Contracaecum aduncum* as described by Berland (loc. cit.). The largest of the three larvae appears to be in the fourth stage, with three lips and a spiny "cactus" tail as described by Berland (loc. cit.). All three larvae have tubular gonads containing loosely packed cells. Well-developed genital tubules were previously reported in third-stage larvae by Berland (loc. cit.). Prior to this report the fourth stage was believed to occur only in the definitive host (Berland, loc. cit.). It is possible that the cuticle of the third stage was removed mechanically during dissection of the larva from the mesoglea of the host and not actively through a molt. However, adult *Contracaecum* have been found in a prawn, *Pandalus borealis* Kroyer, by Margolis and Butler (1954, J. Parasit. 40: 649–655).

According to Hyman (1940, The Invertebrates Vol. 1, McGraw-Hill, New York, 726 p.), the hydromedusae are carnivorous, ingesting crustaceans, polychaetes, ctenophores, other medusae, and small fish, all known hosts of larval *Contracaecum*. Because of their feeding habits, these small planktonic jellyfish may be reservoirs of infection for plankton-feeding fish. *Contracaecum* larvae occurring along the similarly colored radial canals can be easily overlooked. This may account for the paucity of reports of infection.

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Research Note

Age Resistance of Lambs to Single Inoculation with *Haemonchus contortus*

Acquired resistance to reinfection with *Haemonchus contortus* is apparently directly related to age of lamb at time of primary inoculation or vaccination (Manton et al., 1962, Res. Vet. Sci. 3: 308–314; Urquhardt et al., 1966, Am. J. Vet. Res. 27: 1645–1648). The critical age appears to be about 8 months. Although age resistance per se to primary inoculation was shown in cattle (Herlich, 1960, J. Parasit. 46: 392–397) and has been shown in a number of small animal–nematode parasite relationships (reviewed in Herlich, 1960, loc. cit.), no similar evidence has been reported for sheep. This report presents the results of two experiments to determine the effect of lamb age on resistance to primary inoculation with *H. contortus*.

Purebred polled Dorset lambs were raised under conditions which prevented extraneous infections with nematode parasites other than *Strongyloides papillosus*. Postweaning they were maintained in separate stilt pens until they were killed. They were fed a pelleted alfalfa hay ration ad lib.

Larvae were obtained by culturing feces from lambs infected with the BPL strain of *H. contortus*. Larval inocula were quantitated by the method of Colglazier, Kates, and Enzie (1969, Proc. Helm. Soc. Wash. 36: 68–74), and were administered orally in water via syringe to the experimental lambs.

Fecal samples for egg counts were collected days 25 and 26 postinoculation. Lambs were killed on day 26, and standard laboratory procedures were used to recover and enumerate the numbers of *H. contortus*. Worm and nematode egg count data were transformed to log X and subjected to analysis of variance for significance. Treatment means were separated by Kramer's modification of Duncan's multiple range test (Kramer, 1956, Biometrics 12: 307–310).

Experiment 1: 18 lambs were divided into four age groups as follows: 3 lambs, 3 months; 5 lambs, 6 months; 5 lambs, 9 months; 5 lambs, 21 months. All lambs were inoculated with 12,375 ± 570 (95% Confidence Interval = standard error ×2.26, t at 9 degrees of freedom) infective *H. contortus* larvae.

Experiment 2: 20 lambs were divided into four age groups as follows: 5 lambs, 3 months; 5 lambs, 12 months; 4 lambs, 23 months; and 6 lambs, 32 to 35 months. All lambs were infected with 12,375 ± 548 (95% Confidence Interval) infective *H. contortus* larvae. One lamb in the 3-month-old group died during the prepatent period of infection; hence, data for that group are based on the surviving four lambs.

Average egg counts and numbers of *H. contortus* recovered at necropsy are presented in Table 1. One fourth-stage larva was recovered in one aliquot of 1/20 of an abomasal washing, all other worms were mature. In Experiment 1, there was a reduction in average egg and worm counts that correlated directly with increased age, though worm numbers in 3-, 6-, and 9-month-old lambs did not differ significantly from each other. The 21-month-old lambs, however, did have significantly fewer worms ($P < 0.05$) than the other age groups. In Experiment 2, all the older groups had significantly fewer worms ($P < 0.05$) than the 3-month-old group, and significantly lower egg counts.

Table 1. Average egg counts and numbers of *Haemonchus contortus* recovered from lambs of different age groups.

Age group (months)	No. lambs	Egg counts (epg)	No. worms
Experiment 1, 1972 (12,375 ± 570 larvae)*			
3	3	21,690a†	6,444a†
6	5	13,400a	5,654a
9	5	5,283a	4,484a
21	5	1,596b	2,333b
Experiment 2, 1973 (12,375 ± 548 larvae)*			
3	4	14,675a	2,722a
12	5	1,100b	362b
23	4	4,608b	1,081c
32–35	6	1,164b	394b

* Larvae/lamb ± Confidence Interval (95%).
† Means followed by a common letter do not differ ($P < 0.05$).

ROBERT A. KNIGHT AND DELOYCE RODGERS
United States Department of Agriculture
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PRESENTATION

1973 Anniversary Award of The Helminthological Society of Washington

478th Meeting, 16 November 1973

Dr. Elvio H. Sadun

Tonight it is my privilege to present the 1973 Anniversary Award of The Helminthological Society of Washington. The recipient of the award was unanimously and enthusiastically chosen by your Committee on Awards and fully supported by the Executive Committee. He is, of course, a distinguished scientist, but he is also well known for his personal charm and his highly infectious enthusiasm for studies in which he is involved. He is well-known to us all, Dr. Elvio H. Sadun.

Dr. Sadun was born in Italy and finished basic medical studies in that country before coming to the United States. He received the MS degree from Harvard and the ScD from Johns Hopkins. After spending a couple of years at the University of Arkansas he moved to Tulane University where he remained until 1951. I first heard of Dr. Sadun while I was a graduate student at that institution. His friends at Tulane remain numerous and very firm.

Subsequently, he spent much time in the Far East: Thailand, Japan and Korea. There, I believe, he acquired his real love of trematodes and became intrigued by the problems of diagnosis and host-parasite relationships as expressed immunologically. This has remained the hallmark of his career.

Returning to the United States, Dr. Sadun spent some time with the Communicable Disease Center of the Public Health Service in Georgia and later, in 1954, returned to Japan as Advisor in Parasitology to the 406th Medical Laboratory of the United States Army. Following that tour of duty he returned to this country and until recently was Chief of the Department of Medical Zoology and Special Assistant to the Director of Walter Reed Army Institute of Research on basic research for malaria.

His honorary positions with professional societies, review panels, visiting lectureships, etc. are too numerous to mention. He has been honored the world over. In our country he received the Henry Baldwin Ward Medal of the American Society of Parasitologists and the Secretary of the Army's Award for Exceptional Civil Service.

I consider, however, that Dr. Sadun is really only starting; that in his new position in Africa, he will be an even greater catalyst than he was here and that his personal work will not lag. Thus, it is with all best wishes for future productivity that I am proud to present this Anniversary Award assuring you, Dr. Sadun, of our continued admiration and friendship here in Washington. We hope that on visits to this area you will, when possible, come to the Helminthological Society meetings.

GUILLERMO PACHECO

Awards Committee members: Guillermo Pacheco, Chairman; Lloyd Rozeboom; and John Vetterling.

Dr. Sadun's Acceptance

I am surprised, moved and deeply appreciative of the high honor bestowed upon me. This award comes at a most propitious time, just as I am about to become involved in what may turn out to be the greatest challenge of my professional life: the establishment of a major International Institute of Parasitology, supported by a consortium of Nations.

Some of you may not be fully cognizant of the objectives of the International Laboratory

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for Research on Animal Diseases (ILRAD) which I have been asked to organize and direct. What is fairly unique to this laboratory is the intense focus on two parasitic problems, trypanosomiasis and East Coast Fever. It represents the willingness of a group of specialists spanning an enormous spectrum of intellectual interests, to carry out a coordinated, multidisciplinary intensive program conducted in a single institute located in one of the endemic areas. The combined knowledge and imagination of the biochemist, the genetecist, the immunochemist, the pathologist and the zoologist, in all probability will succeed in understanding and overcoming the problem of antigenic modulation toward the ultimate goal of providing safe and practical vaccines. There is clear evidence that great possibilities exist in this type of cooperation, derived by close association between the several disciplines concerned. Trypanosomiasis and East Coast Fever are still such complex entities that no one can yet seriously present a single approach that is likely to succeed in their control. It is for this reason that we are establishing this laboratory to create a close interaction of experts in various disciplines willing to give their time to understanding the totality of the problem and to interrelate their studies for the contribution to a solution. In the past decades there has been a splitting up of parasitology into increasingly specialized fields. Over-specialization has provoked an interdisciplinary dismemberment. It is now time that we try to capitalize on it so that the opposite process, that of intradisciplinary synthesis can be achieved. By building crosslinks among the diverging lines of different disciplines of scientific research we shall restore unity to the whole. It has always been tempting to believe that simplistic, empirical approaches of stomping the vectors to death may actually work, but I believe that all of us know fully well that only careful accumulation of information gathered scientifically will resolve the long term problem. In any event we shall see. If this multidisciplinary approach to parasitic diseases conducted in an international setting will succeed I believe that it will establish a precedent pointing out a new era for parasitology. If not, the consequences for the future of our discipline may be too grim to contemplate.

As I accept your award I am fully aware of the many uncertainties and road blocks which will be confronting me in the next few years. Therefore, your recognition at this time is accepted as an expression of your confidence that I shall succeed in overcoming the difficulties and in bringing this venture to a felicitous culmination. For my part, I am optimistic that with your continued confidence and with the support from my friends and my family I shall succeed in building this International Institute of Parasitology along the lines that we all have been dreaming about.

In closing let me emphasize that is it from the work conducted by members of this Society that I first received the inspiration to carry out parasitological research. From them I also learned to use some of the tools needed for it. Twenty-six years ago, I presented my first paper (it was on immunity to *Ascaridia galli*) before this Society. I was so intimidated by the presence of illustrious parasitologists who had been the object of my veneration that one of your members, Dr. Otto, thought it prudent to let me imbibe a couple of drinks before the meeting, just to get enough courage. Since then there have been many meetings shared with many of you, in the United States as well

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as in some of the far away parts of the world. These memories add to the gratitude which I owe to you for having chosen me for this award. But, even more important than this recognition is the realization that 26 years ago, I met here many strangers who now I can call with pride and affection, my friends. Thank you.

New Book Translation

BAUER, O. N., V. A. MUSSELIUS, YU A. STRELKOV. 1969. Diseases of Pond Fishes (Bolezni prudovykh ryb). Izdatel'stvo "Kolos," Moscow. (English transl. NTIS, U. S. Dept. Commerce, Springfield, Virginia 22151, 220p.)

This book was written by three leading fish disease researchers of the U.S.S.R. The seven chapters include general information, prevention and control, diseases (viral, bacterial, fungal, protozoan, helminth, crustacean, non-communicable, unknown etiology), and methods of fish examination. Also included is an appendix of the main drugs and chemicals used in the control of salmonid and "warm water" fish diseases. The use of antibiotics in the isolation of viruses and fungi was omitted. The text is well illustrated with drawings and some photographs.

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